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Studies on Cirrhosis of the Liver

III. Liver Biopsy and Liver Function Tests in Alcoholics

By

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The relation between alcoholism and liver cirrhosis has been pointed out from old times. Recently there is a growing conviction that alcoholics are liable to develop choline deficiency, which induces fatty liver and liver cirrhosis. The fact that fatty metamorphosis, fibrosis, and hepatic dysfunction are observed in alcoholics[1)-5)] supports this hypothesis. In this country, however, there are some investigators who doubt of this opinion from their studies on liver cirrhosis in necropsied materials.[6)]

For the purpose of investigating this problem liver biopsies and liver function tests were carried out in alcoholics. The results of the investigation are described in the present paper.

Experimental

Material and Methods

Six alcoholics who entered the Department of Psychiatry of Tohoku University for the purpose of Antabuse treatment, one who entered the same department with delirium tremens, and seven who were found by the present authors in mass examination of inhabitants and outpatient examination were investigated. Patients who were admitted with the complaints of liver diseases were excluded.

The investigation included previous history of alcoholic intake and liver and biliary diseases, dietary history, physical examination, liver function tests, and liver biopsies.

Dietary history before the patients abstained from alcohol was investigated, and the intake of protein, fat, carbohydrate, and calories were calculated using a food analysis table.

The liver function tests carried out and their normal values are as follows: 1) icterus index (≦10), 2) 30 minutes bromsulphalein retention test (≦10%), 3) thymol turbidity test (≦4 units) 4) thymol flocculation test (≦+), 5) cephalin cholesterol flocculation test (≦+), 6) Gros's test (≦+), and 7) Wallace-Diamond's urine urobilinogen test (≦×10).

Biopsies were performed within 2 weeks after the last alcoholic intake with two exceptions. Of these two biopsies, one was done 1 month after the last alcoholic intake, and the other 3 months. Biopsy was repeated in one patient 75 days after receiving dietary treatment. Fatty metamorphosis was considered to be positive when fatty cysts were clearly demonstrated in the sections stained with hematoxylin and eosin, and it was classified as follows: + or slight when fatty cysts occupied less than one-third of the sections, ++ or moderate when fatty cysts occupied one-third to two-third, and +++ or severe when fatty cysts occupied more than two-third.

For the controls for the liver function tests factory workers in Sendai who were investigated by the same methods were used, and for the controls for dietary history male factory workers in Sendai and peasants in Yamagata Prefecture of the age of 20 years or more were used.

Observations

The observations are summarised in Table I. The alcoholics investigated were all males. Ages ranged from 23 to 64 years with an average age of 38.9 years. A past history consistent with gall-bladder disease was elicited in two instances. An antecedent history of jaundice was not obtained from the alcoholics.

The amount of average daily alcoholic intake, which was calculated in *sake* (Japanese fermented liquor made from rice containing 15% alcohol) equivalent, was 1 to 16.5 *go* (1 *go*=180 cc), with an average of 7.1 *go*. The period of alcoholic intake was 4 to 36 years, with an average of 16.6 years. When the alcoholics who entered the Psychiatric Department were compared with those who were found in the outpatient and mass examination, the amount of daily alcoholic intake was 7.6 *go* in the former and 5.3 *go* in the latter, and the duration of alcoholic intake was 18.6 years in the former and 14.7 years in the latter. Thus the alcoholic consumption of the former was severer than the latter.

The intakes of protein, fat, carbohydrate, and calories are shown in Table 2. The daily protein intake in 46.1% of the alcoholics was less than 55 g. (Table III). When 46.1% was compared with 8.6% (peasants) or 2.4% (factory workers) of the controls, the differences were both statistically significant ($P<0.0001$). The ratio of protein to total caloric intake

TABLE I

Clinical, Functional and Histological Observations on Alcoholics

Case No.	Age, year	Sex	Source*	Alcoholic intake: Average daily intake**, *go*†	Alcoholic intake: Duration, year	Alcoholic intake: Interval,‡ day	Daily protein intake, g.	Previous history of cholelithiasis	Liver: Enlargement	Liver: Tenderness	Liver function test: Icterus index	Liver function test: B.S.P., %	Liver function test: T.T.T., unit	Liver function test: T.F.T.	Liver function test: C.C.F.	Liver function test: Gros's test	Liver function test: Urobilinogen	Liver biopsy: Fatty metamorphosis	Liver biopsy: Fibrosis	Liver biopsy: Focal necrosis	Liver biopsy: Cellular infiltration in stroma
1	35	M	P	6.5	10	11	43	+	−	−	6	5	2	−	++	−		+	(+)	−	−
2	46	M	P	1.5	25	30	51	−	−	−	6	3	2	−	+	−	× 1	−	−	−	−
3	31	M	P	5.0	12	7	60	−	−	−		5	1					−	−	−	−
4	23	M	P	7.0	5	12	83	−	+	−	4	10	3	−	−	−	× 5	−	−	−	−
5	44	M	P	10.0	22	14	50	−	−	−	8	18	2	−	−	±		+	++	+	+
6	58	M	P	16.5	28	0	47	−	−	+	13	30	2	++	−	+	×10	++	±	−	±
7	48	M	P	6.7	23	6	34	−	+	+	7	20	1	−	−	−	× 1	+	+	+	−
8	40	M	M	5.0	20	<5	37	−	−	−	13	5	2	+	−	±	×10	±	−	−	++
9	64	M	M	10.0	36	180		−	−	−	9	15	1	−	−	±	×10	±	++	−	++
10	38	M	M	1.0	11	4	74	−	−	−	7	16	1	−	+	−	×20	±	−	−	−
11	26	M	O	7.7	4	5	64	−	−	−	7	5	1	−	−	−	× 1	−	−	−	−
12	28	M	O	4.6	8	2	95	+	+	+	8	3	2	+	−	−	× 5	−	−	−	−
13	23	M	O	5.0	7	7	60	−	−	+	9	4	1	−	−	−	× 5	−	−	−	−
14	41	M	O	3.5	17	1	82	. −	−	−	9		3	−	−	−	× 1	−	−	−	−

* P expresses the Psychiatric Department, M : mass examination of inhabitants ; O : outpatient examination. ** Calculated in *sake* (15% alcohol) equivalent. † 1 *go*=180 cc. ‡ Interval between the last alcoholic intake and liver biopsy.

in the alcoholics was markedly decreased as compared with that in the controls, since caloric intake in the alcoholics was larger than the controls owing to the calories of alcohol, though the intake of carbohydrate in the alcoholics was smaller than that in the controls.

Epigastric pain was observed in one patient. Liver enlargement was noted in three cases, and liver tenderness in four. None of the cases showed jaundice, vascular spider, gynecomastia, or ascites.

The results of liver function tests are shown in Table 4. The most frequent biochemical abnormality was bromsulphalein retention. Five of thirteen cases (38.4%) showed abnormal dye retention, whereas only 6.3% of the controls showed abnormal value. The difference was statistically significant (P=0.015). Icterus index was abnormal in 15.4%, and urine urobilinogen, thymol turbidity test, thymol flocculation test, cephalin

TABLE II

Average Daily Intake of Protein, Fat and Carbohydrate in Alcoholics and Controls

	Protein g.	Fat g.	Carbohydrate g.	Calorie Cal.	Protein/Calorie
Alcoholics	60.0	22.6	254.0	2766.7	0.0233
Controls (Peasants)	71.2	20.5	389.4	2016.4	0.0353
Controls (Factory workers)	82.7	31.5	369.6	2242.0	0.0369

TABLE III

Daily Protein Intake in Alcoholics and Controls

	No. of cases	No. of cases whose daily protein intake was less than 55 g.	
Alcoholics	13	6	(46.1%)*
Controls (Peasants)	301	26	(8.6%)*
Controls (Factory workers)	167	4	(2.4%)*

* The differences between the alcoholics and the controls are statistically significant ($P<0.0001$).

TABLE IV

Liver Function Tests in Alcoholics and Controls

	Alcoholics			Controls		
	No. of cases	No. of cases showing abnormal value		No. of cases	No. of cases showing abnormal value	
Icterus index	13	2	(15.4%)	59	1	(1.7%)
B. S. P.	13	5	(38.4%)*	32	2	(6.3%)*
T. T. T.	14	0	(0%)	94	1	(1.1%)
T. F. T.	13	1	(7.7%)	94	7	(7.4%)
C. C. F.	13	1	(7.7%)	83	2	(2.4%)
Gros's test	13	1	(7.7%)	82	0	(0%)
Urobilinogen	11	1	(9.1%)	216	8	(3.7%)

* The difference is statistically significant ($P=0.015$).

cholesterol flocculation test, and Gros's test were abnormal in 0 to 9.1%. The differences between these incidences and those in the controls were not statistically significant.

Liver biopsies revealed fatty metamorphosis in 4 cases (28.6%) (Fig. 1). The degree of fatty metamorphosis was estimated to be severe

in one and slight in three. Fibrosis of the liver was observed in 4 cases (28.6%) (Fig. 2). It was moderate in two and slight in two. One with slight fibrosis had had the previous history of gall-bladder disease, and the fibrosis was considered to be of biliary origin. If this case was excluded,

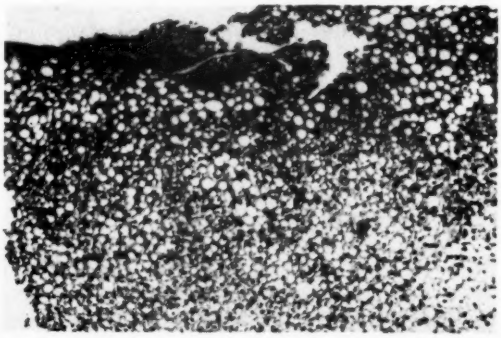

Fig. 1. Liver biopsy specimen of Case 6, showing marked fatty metamorphosis. Van Gieson's stain. ×69.

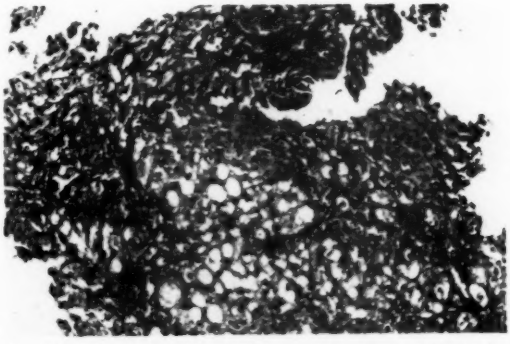

Fig. 2. Liver biopsy specimen of Case 5, showing moderate fibrosis and slight fatty metamorphosis. Cellular infiltration in stroma and focal necrosis are also observed. Between the walls of fatty cysts collagenous fibers are present. Van Gieson's stain. ×68.

fibrosis was present in three out of fourteen cases (21.4%). In one case with moderate fibrosis, collagenous fibers were observed between the walls of fatty cysts, and this finding seemed to suggest that at least one part of fibrosis originated directly from fatty cysts. Focal necrosis was noticed in two cases with fibrosis, and cellular infiltration in stroma was found in two with fibrosis and in one without fibrosis. The second biopsy, which was performed 75 days after the first biopsy in one case, revealed the disappearance of fatty metamorphosis and no change in fibrosis.

Fatty metamorphosis and fibrosis, which are the most principal changes in alcoholics, were both observed in two cases; only fatty metamorphosis was found in two cases, and only fibrosis in one. The last was one in whom liver biopsy was performed 3 months after the last drinking, and it was conceivable that fatty metamorphosis had disappeared during this period. Hence, of 14 cases five (35.7%) showed fatty metamorphosis or fibrosis of the liver which seemed to be the result of the former.

In regard to the relation between histological changes and alcoholic intake, it was noteworthy that all the 5 cases who showed fatty metamorphosis or fibrosis had the history of drinking 6. 5 *go* or more *sake* daily for 10 years or more and their protein intake was less than 55 g., and that none of the cases who did not show fatty metamorphosis or fibrosis drank to such a degree.

The relations between histological changes (fatty metamorphosis or fibrosis) and the amount of daily alcoholic intake, the duration of alcoholic intake, or daily protein intake are shown in Tables 5, 6, and 7. Histologi-

TABLE V

Relation Between Daily Alcoholic Intake and Histological Changes (Fatty Metamorphosis or Fibrosis) or Bromsulphalein Test

	Positive histological change	Negative histological change	Bromsulphalein test >10%	Bromsulphalein test ≦10%
Daily alcoholic intake<6.5 *go*	0*	7	1	5
Daily alcoholic intake≧6.5 *go*	5*	2	4	3

* The difference is statistically significant (P=0.01).

TABLE VI

Relation Between Duration of Alcoholic Intake and Histological Changes (Fatty Metamorphosis or Fibrosis) or Bromsulphalein Test

	Positive histological change	Negative histological change	Bromsulphalein test >10%	Bromsulphalein test ≦10%
Duration of alcoholic intake<10 years	0	4	0	4
Duration of alcoholic intake≧10 years	5	5	5	4

cal changes were observed more frequently in the alcoholics whose daily alcoholic intake was 6.5 *go* or more than in those whose alcoholic intake was less than 6.5 *go*, and in alcoholics whose protein intake was less than

TABLE VII

Relation Between Daily Protein Intake and Histological Changes (Fatty Metamorphosis or Fibrosis) or Bromsulphalein Test

	Positive histological change	Negative histological change	Bromsulphalein test >10%	Bromsulphalein test ≦10%
Daily protein intake <55 g.	4*	2	3	3
Daily protein intake ≧55 g.	0*	7	1	5

* The difference is statistically significant (P=0.02).

TABLE VIII

Relation Between Histological Changes (Fatty Metamorphosis or Fibrosis) and Bromsulphalein Test

	Positive histological change	Negative histological change
Bromsulphalein test >10%	4*	1
Bromsulphalein test ≦10%	1*	7

* The difference is statistically significant (P=0.03).

55 g. than in those whose ptoein intake was 55 g. or more. The differences were statistically significant (P=0.01 and 0.02). However, there was no statistically significant difference between the alcoholics who drank for 10 years or more and those who drank for less than 10 years.

The differences of incidences of positive bromsulphalein test attributable to the amount of alcoholic intake, the duration of alcoholic intake, and daily protein intake were not statistically significant.

Bromsulphalein test was well correlated with the histological changes (P=0.03) (Table VIII).

Discussion

With regard to liver biopsies in alcoholics there are the reports of Chalmers *et al.*,[1] Sanes *et al.*,[2] and Seife *et al.*[3] However, it is not easy to compare the present observations with the results of these reports, since the degree of alcoholic intake seems to be not identical in each investigation. Of these reports that of Seife *et al.* is similar to the present observations in respect to the duration of alcoholic intake and the results of liver function tests, although it is uncertain whether the amount of daily alcoholic intake is similar or not. According to Seife *et al.* the first biopsies which were done within 24 hours of admission revealed liver fibrosis in

20%, fatty metamorphosis in 72%, and focal necrosis in 9%. The incidences of liver fibrosis and focal necrosis are in agreement with those in the present observations. There is, however, some difference of incidence of fatty metamorphosis between the report of Seife *et al.* and the present observations. The difference is considered to be due to the fact that the interval between the last drinking and biopsy is longer in the present observations than that in the report of Seife *et al.* and that in the present observations the degree of alcohol consumption in the alcoholics who were found in the outpatient and mass examination is generally mild. Fatty metamorphosis was present in 57.2% of the alcoholics who entered the psychiatric department. The incidence is nearly the same as that in the second biopsies of Seife *et al.* (56.0%).

It is in accordance with the results of Voegtlin *et al.*,[4] Seife *et al.*,[3] and Nadeau *et al.*[5] that the most frequent biochemical abnormality was bromsulphalein retention. The increase of serum bilirubin, which is reported by Voegtlin *et al.*[4] and Seife *et al.*,[3] was found in 15.4% in the present observation. However, the difference of incidence between the alcoholics and the controls was not statistically significant. Serum turbidity and flocculation tests showed abnormal values infrequently in accord to the results of Voegtlin *et al.*

Thus there is no marked difference of the results of liver function tests and liver biopsies in alcoholics between the American investigators and the present authors. This fact as well as the clinical experiences with fatty metamorphosis indicates that the alcoholics in this country develop fatty liver and fatty liver cirrhosis similarly.

It is noteworthy that the histological examination revealed the close relationship between fatty cysts and fibrosis in one case. It appears to indicate that in fatty liver cirrhosis of man at least one part of fibrosis is the result of the collapse of fatty cysts as described by Hartroft.[7]

With respect to the relation between histological changes and mode of alcoholic intake, histological changes are reported to be more frequently found in steady drinkers than in the intermittent drinkers.[1),3)] The present observations clearly demonstrate that the more daily alcoholic intake and the less protein intake, the more frequently fatty metamorphosis or fibrosis observed.

Summary and Conclusions

1. Liver biopsy, liver function tests, and nutritional investigation were carried out in 14 alcoholics. Those who entered the hospital with the complaints of liver diseases were excluded. Both fatty metamorphosis and fibrosis were observed in two cases, fatty metamorphosis in two, and fibrosis in one. Focal necrosis was noticed in two cases, and cellular in-

filtration in stroma in three.

2. Bromsulphalein test was abnormal in 38% of the alcoholics and in 6.3% of the controls. The difference was statistically singnificant. There were no statistically significant differences of incidences of abnormal values in icterus index, thymol turbidity test, thymol flocculation test, cephalin cholesterol flocculation test, Gros's test, and urine urobilinogen between the alcoholics and the controls.

3. The intakes of protein and carbohydrate in the alcoholics were decreased and total caloric intake including calories of alcohol was increased as compared with those in the controls.

4. With regard to the relation between histological changes and alcoholic intake, all the cases who showed fatty metamorphosis or fibrosis had the history of drinking 6.5 *go* or more *sake* daily for 10 years or more and their protein intake was less than 55 g., and none of those who did not show fatty metamorphosis drank to such a degree. The more daily alcoholic intake and the less protein intake, the more frequently fatty metamorphosis or fibrosis observed.

Bromsulphalein test was well correlated with histological changes.

The authors wish to express their gratitude to Professor Toshimi Ishibashi, Director of the Psychiatric Department, Tohoku University, for all possible help in providing materials.

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The Erythrocytes Sensitized with Tuberculin-Polysaccaride in the Circulating Blood:—Its Diagnostic Application for Early Stage of Tuberculosis in Children*

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In tuberculous cases of children, the erythrocytes sensitized with tuberculin-polysaccharide were detected by means of the agglutination reaction between the erythrocytes and the immune serum prepared in the following way. Sheep were treated by repeated injection of killed tubercle bacilli, until the serum obtained from same was able to cause an agglutination with the human erythrocytes sensitized with 0.178 mg% solution of tuberculin-polysaccharide.

The cases of tuberculosis with sensitized erythrocytes in the circulating blood were more frequently found in the age under 3 years than in the older children, while the cases with the antibodies against tuberculin or tuberculin-polysaccharide in the serum occurred more frequently in older children. This relation between the age on one side and the phenomenon between the sensitized erythrocytes in the blood and the antibodies against tuberculin or tuberculin-polysaccharide on the other held good, irrespective[2] of the results of the Mantoux test (using old tuberculin of a dilution 1:2000).

As the factors contributing to facilitating the sensitization of the erythrocytes with tuberculin-polysaccharide in infants, we want to emphasize the poor formation[1] of the antibody against tuberculin-polysaccharide in infantile age on the one hand and frequent contact with tuberculous patients in the family circle on the other hand.

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Toxic Substances in Cancer

V. Anemia-inducing Substances in the Tissue of Gastric Carcinoma

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The K.I.K. reaction[1] that the gastric juice of a patient with gastric carcinoma will, if injected intravenously, produce anemia in a rabbit has been studied in our clinic by Harima,[2] Ogata,[3] and others, and the nature of the factor responsible for this reaction is becoming clear. With a view to seeing whether the gastric carcinoma tissue itself contains such a factor, we have undertaken the present work, which essentially consists of fractionating the tissue of gastric carcinoma finely, testing each fraction for the anemia-inducing activity on a rabbit, and tracing the factor. Part of the results obtained will elsewhere be reported in detail by Suzuki,[4] one of the present authors.

Experimental

Preparation of Material

I. Extraction with distilled water

1) *Extraction.* A tissue of gastric carcinoma which was obtained at operation was lightly washed in distilled water to remove blood and mucus, cut into small pieces, dried in a vacuum desiccator, and ground to powder. The dry powder (23 g.) was extracted by stirring with 10 times as much distilled water for 48 hours. The extract was centrifuged, and the residue was further extracted twice, each with 2 liters of distilled water. Of the first extract, 10 cc. was condensed under reduced pressure at a temperature lower than 40°C., and dried to powder (**Cr_I**, yield 125 mg.).

2) *Dialysis.* The remaining first extract and the second and third extracts were each dialyzed against distilled water through cellophane for 48 hours, the dialyzate being changed with distilled water every 8 hours. After dialysis was completed, the dialyzed solution (inner liquid) was condenced under reduced pressure to a syrup, to which was added 5 times as much alcohol. The separated substance was washed with ether and dried to a yellow powder (ND_I, yield 1.3 g.). All the parts of the

dialyzate were pooled, condensed, and dried to a brown muddy substance (**D_I**, yield 2.3 g.).

3) *Fractionation of D_I.* To 2.08 g. of the D_I was added 200 cc. of alcohol little by little with constant agitation. The insoluble part was collected by centrifugation and dried in a desiccator (**$D_{I\text{-alc. ins.}}$**, yield 320 mg.). The solvent was removed from the alcohol-soluble part by evaporation under reduced pressure, and a dry solid substance was obtained (**$D_{I\text{-alc. sol.}}$**, yield 1.62 g.).

To 1.6 g. of the $D_{I\text{-alc. sol.}}$ was added 200 cc. of ether little by little with constant agittation. The insoluble part was collected by centrifugation (**$D_{I\text{-eth. ins.}}$**, yield 1.51 g.). The solvent was removed from the supernatant by evaporation under reduced pressure to a dry solid substance (**$D_{I\text{-eth. sol.}}$**, yield 30 mg.).

II. Extraction with alcohol

1) *Extraction.* A fresh tissue of gastric carcinoma (438 g.) was put in 1.5 liters of alcohol and allowed to stand there for about 2 months. The extract was separated, condensed under reduced pressure, and dried to a yellow-brown muddy substance (10.7 g.). To 1 g. of this substance was added, while agitating at room temperature, a total of 300 cc. of absolute alcohol. The extract obtained was condensed under reduced pressure and dried (**Cr_{II}**, yield 850 mg.).

2) *Dialysis.* Of the Cr_{II}, 830 mg. was mixed with 200 cc. of distilled water. The milky suspension thus formed was dialyzed against distilled water through cellophane for 3 days, with the dialyzate changed with distilled water every 8 hours. After completion of the dialysis, the dialyzed solution was condensed under reduced pressure and dried (**ND_{II}**, yield 490 mg.). All the portions of the dialyzate were pooled and condensed under reduced pressure, and dried to a solid substance (**D_{II}**, yield 300 mg.).

3) *Fractionation of the D_{II}.* Of the D_{II}, 280 mg. was put in 200 cc. of ether, and the mixture was stirred. The insoluble part was collected by centrifugation and dried (**$D_{II\text{-eth. ins.}}$**, yield 230 mg.). By evaporation of ether from the supernatant, a solid substance was obtained (**$D_{II\text{-eth. sol.}}$**, yield 25 mg.).

III. Extraction with saline

1) *Extraction.* The residue after the above-mentioned alcohol extraction was dried in a current of air. To this dried tissue (62 g.) was added 10 times as much 10% saline. After 48 hours' extraction, the extract was made clear by filtration and centrifugation, and was dialyzed against running water for 3 days. The dialyzed solution was condensed under reduced pressure to about 1/10 in volume.

2) *pH* 4.2. The pH of the condensed extract was brought to pH 4.2 with 10% hydrochloric acid. The resulting white flocculent precipi-

tate was collected by centrifugation, put in 30 cc. of distilled water, and dissolved at pH 7.0 (the pH being adjusted with 10% sodium hydroxide). The insoluble part that remained in a small quantity was removed by centrifugation; the pH of the supernatant was adjusted to 4.2; and the resulting precipitate was collected. This process of purification by dissolving and precipitating was repeated further twice. The precipitate finally obtained was dissolved in a small quantity of distilled water, and dialyzed against running water for 3 days. After completion of the dialysis, the dialyzed solution was condensed under reduced pressure to a syrup, to which was added alcohol. The substance thus separated was washed twice with alcohol and once with ether, and dried (**Ppt** $_{pH\ 4.2}$, yield 150 mg.).

3) *pH* 2.0. The supernatant at pH 4.2 and the distilled water washings of the precipitate at pH 4.2 were pooled, and the pH was brought to 2.0 with 10% hydrochloric acid. The precipitate formed was collected by centrifugation after 10 minutes, put in 30 cc. of distilled water, and dissolved at pH 7.0. After removal of the insoluble part by centrifugation, the supernatant was brought to pH 2.0. The precipitate formed was removed by centrifugation. After this process was repeated twice more, the precipitate finally obtained was dissolved in a small quantity of distilled water, and dialyzed against running water for 3 days. After the dialysis, the dialyzed solution was condensed under reduced pressure to a syrup, to which was added alcohol. The substance thus separated was washed with alcohol and ether, and dried (**Ppt** $_{pH\ 2.0}$, yield 130 mg.). The supernatant at pH 2.0 and the washings of the precipitate at pH 2.0 were pooled, brought to pH 5.0, and dialyzed against running water for 3 days. The dialyzed solution was condensed under reduced pressure and dried to powder (**Sp** $_{pH\ 2.0}$, yield 200 mg.).

The residue after the first extraction with 10% saline was reextracted with 10% saline shaking for 48 hours. The extraction was repeated 5 times in all. Each extract thus obtained was fractionated as described above, and all the corresponding fractions were pooled respectively (total yields: Ppt $_{pH\ 4.2}$, 700 mg.; Ppt $_{pH\ 2.0}$, 610 mg.; Sp $_{pH\ 2.0}$, 360 mg.).

4) *Fractionation with ammonium sulfate.* Of the Sp $_{pH\ 2.0}$, 400 mg. was dissolved in 100 cc. of distilled water. Ammonium sulfate was added to the solution to full saturation. The mixture was allowed to stand at room temperature for 3 hours and then centrifuged. The precipitate was dissolved in a small amount of distilled water; this solution and the supernatant were dialyzed against running water for 3 days, condensed under reduced pressure each to a syrup, to which was added alcohol. The substance thus separated from each solution was washed with ether and dried to powder (from the precipitate; **Ppt** $_{(NH_4)_2SO_4}$ 60 mg.: from the

supernatant; **Sp** $_{(NH_4)_2SO_4}$ 160 mg.).

5) *Alcohol fraction of the* $Sp_{(NH_4)_2SO_4}$. Of the Sp $_{(NH_4)_2SO_4}$, 130 mg. was dissolved in 50 cc. of distilled water. To the solution was added an equal volume of alcohol and several drops of alcohol saturated with sodium acetate. The precipitate formed was collected by centrifugation, washed with 50% alcohol, and dried (**Ppt** $_{alc.\ 50\%}$, yield 36 mg.). The supernatant and the washings were pooled, and alcohol was added to a final concentration of 90%. The precipitate thus formed was collected by centrifugation and dried (**Ppt** $_{alc.\ 90\%}$, yield 5 mg.). The supernatant was condensed under reduced pressure and dried (**Sp** $_{alc.\ 90\%}$, yield 16 mg.).

Qualitative Tests

The biuret and Molisch tests were made on the various fractions obtained in the above-mentioned manner from the tissue of gastric carcinoma. The results are shown in Table I.

Control Meterial

The healthy portion of the mucosa obtained from stomachs at oper-

TABLE I

Biuret and Molisch Tests on the Various Fractions

Fraction	Biuret	Molisch
Cr_I	+	+
ND_I	+	+
D_I	+	+
$D_{I\text{-alc. ins.}}$	+	+
$D_{I\text{-alc. sol.}}$	+	+
$D_{I\text{-eth. ins.}}$	−	−
$D_{I\text{-eth. sol.}}$	+	+
Cr_{II}	+	+
ND_{II}	+	±
D_{II}	+	+
$D_{II\text{-eth. ins.}}$	−	−
$D_{II\text{-eth. sol.}}$	±	+
$Ppt_{\ pH\ 4.2}$	++	±
$Ppt_{\ pH\ 2.0}$	++	±
$Sp_{\ pH\ 2.0}$	+	++
$Ppt_{\ (NH_4)_2SO_4}$	++	+
$Sp_{\ (NH_4)_2SO_4}$	+	++
$Ppt_{\ alc.\ 50\%}$	±	++
$Ppt_{\ alc.\ 90\%}$	±	++
$Sp_{\ alc.\ 90\%}$	±	++

ation for gastric or duodenal ulcer was used as the control material. The material was extracted and fractionated in the same manner as the tissue of gastric carcinoma.

Method of Testing for Anemia-inducing Activity

The activity causing anemia in rabbits was tested according to Iwatsuru's new method for the K.I.K. reaction[5] as follows: A 1% solution of the test material in sterilized saline was injected into the ear vein of a rabbit. The number of erythrocytes was determined 1, 2, 3, 4, and 5 hours after injection, and compared with the number before injection. If at least one of those numbers observed 3 to 5 hours after injection was smaller than 90% of the number before injection, the test was judged to be positive to anemia-inducing activity. The amount of injection used for the first test was 1 mg. per kg. body weight of the rabbit. When the test was positive, another test was made at a smaller amount of injection.

Results

The results of the tests with the various fractions of gastric carcinoma and the corresponding fractions of normal gastric mucosa are given in Table II.

Discussion and Conclusions

The aqueous extract of gastric carcinoma was found to have a definitely higher anemia-inducing activity than that of normal gastric mucosa (Table II). This action was lost through dialysis; and a very effective solid substance (effective at a very low concentration like 0.025 mg/kg) was obtained from the dialyzate (Table II). From these findings it may be concluded that an anemiogenic factor is dialyzable and accordingly is a substance of relatively low molecular weight. This fraction is the same one as that demonstrated by Maki[6] to inhibit the activity of the isolated frog heart.

Anemia-inducing activity was demonstrated in the $D_{II\text{-eth. ins.}}$ fraction of the alcohol extract of gastric carcinoma, too (Table II). This fraction agreed with the $D_{I\text{-eth. ins.}}$ fraction of the aqueous extract in its dialyzability, its solubility to alcohol and ether, and its negativeness to either of the biuret and Molisch tests. From these similarities both fractions may be taken to be nearly the same.

The $Sp_{(NH_4)_2SO_4}$ fraction of the 10% saline extract also was effective (0.1 mg/kg), but it was non-dialyzable (Table II). It is thus different from the two fractions mentioned above. When it was further fractioned with alcohol, the fraction that precipitated at a concentration of 90% of

TABLE II

Tests of the Various Fractions of Gastric Carcinoma for Anemia-inducing Activity

Fraction	Gastric carcinoma				Control		
	Amount injected (mg./kg.)	No. of cases	Decrease in no. of erythrocytes	Judgement	No. of cases	Decrease in no. of erythrocytes	Judgement
Cr_I	1	4	16.1	+	4	11.0	+
	0.5				2	4.5	−
ND_I	1	4	9.5	−	4	5.0	−
D_I	1	4	16.1	+	4	8.3	−
	0.1	4	10.5	+			
	0.05	4	12.4	+			
	0.025	4	10.2	+			
	0.01	2	9.2	−			
D_I-alc. ins.	1	2	10.1	+			
D_I-alc. sol.	1	2	10.2	+			
	0.5	2	10.2	+			
	0.05	2	10.3	+			
	0.025	2	0.0	−			
D_I-eth. ins.	1	3	12.0	+			
	0.5	2	10.2	+			
	0.05	2	9.8	−			
D_I-eth. sol.	1	2	10.1	+			
	0.5	2	9.0	−			
Cr_{II}	1	4	13.2	+	4	1.0	−
ND_{II}	1	2	2.1	−			
D_{II}	1	2	12.2	+			
	0.1	2	24.0	+			
D_{II}-eth. ins.	1	2	12.0	+			
	0.5	2	10.3	+			
	0.05	2	7.2	−			
D_{II}-eth. sol.	1	2	7.2	−			
Ppt pH 4.2	1	4	5.1	−	3	7.5	−
Ppt pH 2.0	1	4	6.3	−	3	7.2	−
Sp pH 2.0	1	3	8.2	−	3	4.1	−
Ppt $(NH_4)_2SO_4$	1	4	4.5	−	4	3.9	−
Sp $(NH_4)_2SO_4$	1	3	14.5	+	3	4.7	−
	0.5	2	12.0	+			
	0.1	2	11.2	+			
Ppt alc. 50%	1	4	11.5	+			
	0.1	4	4.0	−			

TABLE II—*Concluded*

Fraction	Gastric carcinoma				Control		
	Amount injected (mg./kg.)	No. of cases	Decrease in no. of erythrocytes	Judgement	No. of cases	Decrease in no. of erythrocytes	Judgement
Ppt alc. 90%	0.1	4	10.2	+			
	0.05	4	12.6	+			
	0.025	2	8.5	−			
Sp alc. 90%	1	4	11.5	+			
	0.1	4	7.5	−			

alcohol was most effective (Table II). Thus, the factor seems to have been concentrated in this fraction.

Further, it was weakly positive to the biuret test and strongly positive to Molisch's (Table I), and seems to be a carbohydrate like the K.I.K. factor I,[7] which is one of the anemia-inducing factors that have been isolated from the gastric juice of patients with gastric carcinoma.

SUMMARY

Various fractions of the tissue of gastric carcinoma and of normal gastric mucosa (as controls) were tested for anemia-inducing activity in rabbits.

1. A crude extract of gastric carcinoma showed a higher anemia-inducing activity than that of normal gastric mucosa.

2. Either of the dialyzable and the non-dialyzable fraction of gastric carcinoma contained an anemia-inducing factor. The factor in the former fraction seems to be a substance of small molecular weight, which is negative to the biuret and Molisch tests, whereas the factor in the latter seems to be a carbohydrate.

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Occurrence of the Erythrocytes Sensitized with Tuberculin-Polysaccharide in the Circulating Blood of the Rabbits with Tuberculous Infection*

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When 40 mg of living tubercle bacilli of Aoyama-B-strain, suspended in 4 cc of saline, were given intratracheally to rabbits, the erythrocytes sensitized with tuberculin-polysaccharide were demonstrated in the circulating blood on the 3rd, 4th and 5th days after the inoculation with the bacilli, but not found after the 6th day, when the antibody against the tuberculin-polysaccharide began to be developed in the serum (Cf. Fig. 1).

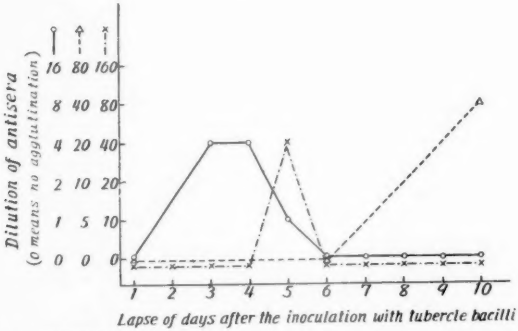
Dilution of antisera
(0 means no agglutination)
16 80 160
8 40 80
4 20 40
2 10 20
1 5 10
0 0 0
1 2 3 4 5 6 7 8 9 10
Lapse of days after the inoculation with tubercle bacilli

Fig. 1. Erythrocytes sensitized with tuberculin, the antibody against tuberculin-polysaccharide in serum, and the tuberculin-polyssacharide in urine in rabbit No. 1 after the intratracheal inoculation of 40 mg. of tubercle bacilli (Aoyama-B-strain).
○—○ means erythrocytes sensitized with tuberculin. △- - - △ means antibody against tuberculin. ×— · — · — × means tuberculin polysaccharide in urine.

The sensitization of erythrocytes with tuberculin-polysaccharide was demonstrated by means of the agglutination between the erythrocytes in question and the immune serum capable of agglutinating the rabbit erythrocytes sensitized with 0.178 mg% solution of tuberculin-polysaccharide.

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* Read at the 12th Meeting of the Aomori Pediatric Society, March 24, 1957, Hirosaki.

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Free Amino Acids in Gastric Juice of Patients with Gastric or Duodenal Ulcer and Gastric Carcinoma, with Special Reference to the Change after Operation

By

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Shimizu[1] in our clinic has studied, by Sawasaki's paper-chromatographic method,[2] the state of occurrence of free amino acids in the gastric juice mainly before operation of surgical patients with gastric or duodenal ulcer and gastric carcinoma. In the work to be reported here, the free amino acids of the gastric juice were collected, by means of ion-exchange resin recommended by Partridge[3][4] and others, with 14 surgical cases of non-gastric diseases, 31 cases of gastric and/or duodenal ulcer, and 68 cases of gastric carcinoma, and examined by a paper-chromatographic method. With gastrectomized cases (29 cases of gastric and/or duodenal ulcer and 43 cases of gastric carcinoma), the free amino acids in the gastric juice before and after operation were compared. More detailed report will be published elsewhere.

Experimental

Test Material

An amount (20 to 30 cc.) of gastric juice was obtained by suction from each patient while hungry early in the morning, filtered through filter paper, and passed through a layer of resin (Amberlite IR-120 of the type H; diameter: 1 cm., height: 50 cm.) at a rate of 1 to 2 cc. per minute. After the layer was washed with distilled water, about 50 cc. of 2 *N* ammonia water was passed through the layer at a rate of 1 to 2 cc. per minute. The eluate thus obtained was condensed under reduced pressure, and dried in a vacuum desiccator to a sloid, and kept as the material for the present experiments.

Paper Chromatography

The test material mentioned above was dissolved in distilled water in

the proportion of 100 mg. per cc. A small amount of this solution was applied to a corner of a sheet of filter paper (Toyo No. 2), and developed by the two-dimensional ascending method. The development was made first with a *n*-butanol-acetic acid-water mixture (4:1:2), then with a phenol-water mixture (4:1) at 20±5°C. until the solvent front advanced 20 cm. After development, the filter paper was dried, over which 0.2% ninhydrin in water-butanol was sprayed; the paper was then heated to reveal colored spots. The kind and color intensity of amino acids were judged by comparison with the standard map prepared in the same manner. In the tables accompanying, " the rate of occurrence " of an amino acid means the rate of the number of cases revealing that amino acid to the total number of cases examined; " negative " (−) in the degree of occurrence means that the corresponding color spot did not appear at all; and " strongly positive " (+) and " positive " (+) mean that the colors of the spots were very strong and intermediate, respectively.

Results

A. Free amino acids in the gastric juice before operation

1) Surgical cases with non-gastric diseases. The results from 14 surgical cases with non-gastric diseases are given in Table I. It will be seen that leucine, alanine, serine and glycine, and glutamic acid occurred in all cases; aspartic acid and histidine, in 12 to 13 cases (85.7–92.9%); and arginine, lysine, cysteine, and phenyl-alanine, in 3 to 6 cases (21.4–42.9%) No other amino acids were recognized. The color intensity of the amino acids was of the order of positive (+).

2) Cases of gastric and/or duodenal ulcer. The results obtained before operation with 31 cases (14 gastric, 14 duodenal, 3 gastric and duodenal cases) are shown in Table I. It will be noticed that tyrosine, which was not found in the cases of non-gastric diseases, occurred in 16 cases (51.6%), 12 amino acids appearing in all. Leucine, valine, glutamic acid, phenyl-alanine, and arginine were strongly positive (+), though in only one case.

Relation with the free hydrochloric acid in the gastric juice: A total of 29 ulcer cases were classified into three groups according to the degree of the acidity of the gastric juice: group 1 (anacidity and hypo-acidity, 0–19 degree); group 2 (normal acidity; 20–40 degree); and group 3 (hyperacidity; 41 degree or higher); and the stage of occurrence of amino acids in each group was compared, with the results as summarized in Table II. There were seen no large differences in the rate of occurrence of amino acids among the groups, except for aspartic acid, phenyl-alanine, and arginine, which showed a little higher rate in groups 2 and 3.

Relation with pyloric obstruction: When 31 ulcer cases were divided

TABLE I

Rate and Degree of Occurrence of Free Amino Acids in the Gastric Juice of Cases of Various Diseases

Amino acid	Healthy stomach (14 cases)			Gastric and/or duodenal ulcer (31 cases)			Gastric carcinoma (68 cases)		
	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%
Leucine	0	14 (100)	0	0	30 (96.8)	1 (3.2)	0	17 (25.0)	51 (75.0)
Valine	0	14 (100)	0	0	30 (96.8)	1 (3.2)	0	19 (27.9)	49 (72.1)
Alanine	0	14 (100)	0	0	30 (96.8)	1 (3.2)	0	26 (38.2)	42 (61.8)
Serine & glycine	0	14 (100)	0	0	31 (100)	0	0	47 (69.1)	21 (30.9)
Glutamic acid	0	14 (100)	0	0	30 (96.8)	1 (3.2)	0	49 (72.1)	19 (27.9)
Phenyl-alanine	11 (78.6)	3 (21.4)	0	18 (58.1)	12 (38.7)	1 (3.2)	28 (41.2)	25 (36.8)	15 (22.1)
Histidine	1 (7.1)	13 (92.9)	0	0	31 (100)	0	1 (1.5)	53 (77.9)	14 (20.6)
Arginine	8 (57.1)	6 (42.9)	0	8 (25.8)	22 (71.0)	1 (3.2)	18 (26.5)	43 (63.2)	7 (10.3)
Aspartic acid	2 (14.3)	16 (85.7)	0	4 (12.9)	27 (81.1)	1 (3.2)	12 (17.6)	51 (75.0)	5 (7.4)
Lysine	10 (71.4)	4 (28.6)	0	21 (67.7)	10 (32.3)	0	29 (42.6)	37 (54.5)	2 (2.9)
Tyrosine	14 (100)	0	0	15 (48.4)	16 (51.6)	0	17 (25.0)	51 (75.0)	0
Proline	14 (100)	0	0	31 (100)	0	0	35 (51.5)	33 (48.5)	0
Cystine	10 (71.4)	4 (28.6)	0	28 (90.3)	3 (9.7)	0	46 (67.6)	22 (32.4)	0
Threonine	14 (100)	0	0	31 (100)	0	0	51 (75.0)	17 (25.0)	0
Peptide	14 (100)	0	0	31 (100)	0	0	46 (67.6)	22 (32.4)	0

into two groups, a group with pyrolic obstruction (9 cases) and a group without the obstruction (22 cases), no significant differences in the state of occurrence of amino acids were seen as a whole between the two groups, though some individual amino acids showed more or less differences.

3) Cases of gastric carcinoma. The state of occurrence of free amino acids in the gastric juice of 68 cases of gastric carcinoma (15 total gastrectomy, 43 subtotal gastrectomy, and 10 gastrectomy-impossible cases) is shown in Table I. It will be seen that, in addition to the amino acids occurring in the gastric ulcer cases, proline, threonine, and a peptide

TABLE II

Relation of Gastric Juice Acidity with State of Occurrence of Free Amino Acids in Gastric Juice of Gastric and/or Duodenal Ulcer Cases

Amino acid	Group I*			Group II†			Group III‡		
	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%
Leucine	0	9 (100)	0	0	8 (88.9)	1 (11.1)	0	11 (100)	0
Valine	0	9 (100)	0	0	8 (88.9)	1 (11.1)	0	11 (100)	0
Alanine	0	9 (100)	0	0	8 (88.9)	1 (11.1)	0	11 (100)	0
Serine & glycine	0	9 (100)	0	0	9 (100)	0	0	11 (100)	0
Glutamic acid	0	9 (100)	0	0	8 (88.9)	1 (11.1)	0	11 (100)	0
Phenyl-alanine	8 (88.9)	1 (11.1)	0	3 (33.3)	5 (55.6)	1 (11.1)	6 (54.5)	5 (45.5)	0
Histidine	0	9 (100)	0	0	9 (100)	0	0	11 (100)	0
Arginine	4 (44.4)	5 (55.6)	0	2 (22.2)	6 (66.7)	1 (11.1)	2 (18.1)	9 (81.9)	0
Aspartic acid	2 (22.2)	7 (77.8)	0	1 (11.1)	8 (88.9)	0	0	11 (100)	0
Lysine	6 (66.7)	3 (33.3)	0	6 (66.7)	3 (33.3)	0	7 (63.6)	4 (36.4)	0
Tyrosine	3 (33.3)	6 (66.7)	0	3 (33.3)	6 (66.7)	0	7 (63.6)	4 (36.4)	0
Proline	9 (100)	0	0	9 (100)	0	0	11 (100)	0	0
Cystine	9 (100)	0	0	8 (88.9)	1 (11.1)	0	10 (90.9)	1 (9.1)	0
Threonine	9 (100)	0	0	9 (100)	0	0	11 (100)	0	0
Peptide	9 (100)	0	0	9 (100)	0	0	11 (100)	0	0

* Anacidity and hypoacidity, 0–19 degree, 9 cases. † Normal acidity, 20–40 degree, 9 cases. ‡ Hyperacidity, 41 degree or higher, 11 cases.

newly appeared in 17 to 33 cases (25.0–48.5%), 14 amino acids and a peptide being found in all. Furthermore, the rate of occurrence tended to increase; thus, leucine, valine, alanine, serine and glycine, and glutamic acid were strongly positive in 19 to 51 cases (27.9–75.0%).

Relation with the acidity of the gastric juice: When 67 cases were divided into two groups, a group showing a total acidity lower than 19 (38 cases) and a group showing a total acidity higher than 20 (29 cases), no

TABLE III

Relation of Size of Tumors with State of Occurrence of Free Amino Acids in Gastric Juice of Gastric Carcinoma Cases

Amino acid	Smaller*			Medium†			Larger‡		
	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%
Leucine	0	4 (66.7)	2 (33.3)	0	8 (18.6)	35 (81.4)	0	1 (11.1)	8 (88.9)
Valine	0	3 (50.0)	3 (50.0)	0	10 (23.3)	33 (76.7)	0	2 (22.2)	7 (77.8)
Alanine	0	3 (50.0)	3 (50.0)	0	22 (51.2)	21 (48.8)	0	2 (22.2)	7 (77.8)
Serine & glycine	0	6 (100)	0	0	32 (74.4)	11 (25.6)	0	5 (55.6)	4 (44.4)
Glutamic acid	0	6 (100)	0	0	34 (79.1)	9 (20.9)	0	4 (44.4)	5 (55.6)
Phenyl-alanine	3 (50.0)	3 (50.0)	0	20 (46.5)	13 (30.2)	10 (23.2)	3 (33.3)	4 (44.4)	2 (22.2)
Histidine	0	6 (100)	0	1 (2.3)	34 (79.1)	8 (18.6)	0	6 (66.7)	3 (33.3)
Arginine	2 (33.3)	4 (66.7)	0	11 (25.6)	27 (62.8)	5 (11.6)	3 (33.3)	6 (66.7)	0
Aspartic acid	0	6 (100)	0	10 (23.3)	32 (75.4)	1 (2.3)	0	7 (77.8)	2 (22.2)
Lysine	4 (66.7)	2 (33.3)	0	20 (46.5)	22 (51.2)	1 (2.3)	3 (33.3)	6 (66.7)	0
Tyrosine	4 (66.7)	2 (33.3)	0	12 (27.9)	31 (72.1)	0	1 (11.1)	8 (88.9)	0
Proline	4 (66.7)	2 (33.3)	0	23 (53.5)	20 (46.5)	0	4 (44.4)	5 (55.6)	0
Cystine	4 (66.7)	2 (33.3)	0	31 (72.1)	12 (27.9)	0	3 (33.3)	6 (66.7)	0
Threonine	6 (100)	0	0	31 (72.1)	12 (27.9)	0	6 (66.7)	3 (33.3)	0
Peptide	4 (66.7)	2 (33.3)	0	33 (76.7)	10 (23.3)	0	7 (77.8)	2 (22.2)	0

* Smaller than 5 cm. in diameter, 6 cases. † 5–9 cm. in diameter, 43 cases. ‡ Larger than 10 cm. in diameter, 9 cases.

significant differences in the state of occurrence of amino acids were noticed between the two groups.

Relation with histologic findings: Between two groups of gastric carcinoma (57 cases), a group of carcinoma adenomatosum (29 cases) and a group of carcinoma solidum (28 cases), there were no noticeable differences in the state of occurrence of free amino acids in the gastric juice before operation.

Relation with the size of tumors: When 58 cases of gastric carcinoma

TABLE IV

Comparison between the Preoperative and Postoperative States of Occurrence of Gastric Juice Free Amino Acids in Cases of Gastrectomy for Gastric and/or Duodenal Ulcer and Gastric Carcinoma

Amino acid	Gastric and/or duodenal ulcer (29 cases)						Gastric carcinoma (43 cases)					
	Before operation			After operation			Before operation			After operation		
	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%	− ( )%	+ ( )%	++ ( )%
Leucine	0	28 (96.6)	1 (3.4)	0	29 (100)	0	0	10 (23.3)	33 (76.7)	0	39 (90.7)	4 (9.3)
Valine	0	28 (96.6)	1 (3.4)	0	29 (100)	0	0	11 (25.6)	32 (74.4)	0	40 (93.0)	3 (7.0)
Alanine	0	28 (96.6)	1 (3.4)	0	29 (100)	0	0	18 (41.9)	25 (58.1)	0	39 (90.7)	4 (9.3)
Serine & glycine	0	29 (100)	0	0	29 (100)	0	0	33 (76.7)	10 (23.3)	0	41 (95.3)	2 (4.7)
Glutamic acid	0	28 (96.6)	1 (3.4)	0	29 (100)	0	0	33 (76.7)	10 (23.3)	0	41 (95.3)	2 (4.7)
Phenylalanine	17 (58.7)	11 (37.9)	1 (3.4)	23 (79.3)	6 (20.7)	0	21 (48.8)	14 (32.6)	8 (18.6)	32 (74.4)	11 (25.6)	0
Histidine	0	29 (100)	0	3 (10.3)	26 (89.7)	0	1 (2.3)	35 (81.4)	7 (16.3)	5 (11.6)	37 (86.1)	1 (2.3)
Arginine	8 (27.6)	20 (69.0)	1 (3.4)	14 (48.3)	15 (51.7)	0	12 (27.9)	28 (65.1)	3 (7.0)	28 (65.1)	15 (34.9)	0
Aspartic acid	4 (13.8)	25 (86.2)	0	6 (20.7)	23 (79.3)	0	7 (16.3)	34 (79.1)	2 (4.7)	14 (32.6)	29 (67.4)	0
Lysine	19 (65.5)	10 (34.5)	0	25 (86.5)	4 (13.8)	0	22 (51.2)	21 (48.8)	0	33 (76.7)	10 (23.3)	0
Tyrosine	13 (44.8)	16 (55.2)	0	23 (79.3)	6 (20.7)	0	13 (30.2)	30 (69.8)	0	26 (83.7)	7 (16.3)	0
Proline	29 (100)	0	0	29 (100)	0	0	24 (55.8)	19 (44.2)	0	40 (93.0)	3 (7.0)	0
Cystine	26 (89.7)	3 (10.3)	0	27 (93.1)	2 (6.9)	0	29 (67.4)	14 (27.9)	0	35 (81.4)	8 (18.6)	0
Threonine	29 (100)	0	0	29 (100)	0	0	31 (72.1)	12 (27.9)	0	43 (100)	0	0
Peptide	29 (100)	0	0	29 (100)	0	0	33 (76.7)	10 (23.3)	0	43 (100)	0	0

(43 subtotal gastrectomy, 15 total gastrectomy cases) were classified into 3 groups according to the size of tumors (smaller than 5 cm. in diameter: 6 cases; 5–9 cm.: 43 cases; larger than 10 cm.: 9 cases), it was found as shown in Table III that the state of occurrence of free amino acids in the gastric juice before operation was relatively stronger with increase of tumor size.

B. Free amino acids in the gastric juice after operation

1) Cases of gastric and/or duodenal ulcer. Two to three weeks after gastrectomy with 29 cases of gastric or duodenal ulcer (13 gastric, 14 duodenal, 2 gastric and duodenal ulcer cases), gastric juice was obtained from the remaining stomachs, and the state of occurrence of free amino acids was examined. By comparison with the results obtained from the same cases before operation (Table IV), it was found that the rate of occurrence of each free amino acid was nearly the same as that before operation, but the state of occurrence showed a slight tendency to fall, generally resembling the state as seen in the cases with the healthy stomach.

2) Cases of gastric carcinoma. With 43 gastric carcinoma cases, examination of free amino acids in the gastric juice of the remaining stomach was made two to three weeks after gastrectomy, gave the following results. By comparison of the results with those obtained before operation with the same cases, it was found that there appeared a remarkable depression both in the rate and in the degree of occurrence of free amino acids, especially of theose amino acids which were strongly positive (++) before operation. Thus, leucine, valine, alanine, serine and glycine, glutamic acid, and histidine showed a strong positiveness (++) in only 1 to 4 cases (2.3–9.3%) postoperatively; and threonine and the peptide did not occur after operation. Generally, this postoperative state of occurrence of free amino acids in the gastric carcinoma cases may be said to be similar to the preoperative state in the ulcer cases.

Summary

The free amino acids in the gastric juice of patients with gastric and/or duodenal ulcer and gastric carcinoma were collected by means of ion exchange resin and examined by a paper-chromatographic method.

1. In the gastric juice of healthy stomachs occurred 11 free amino acids. The degree of occurrence, however, was of the order of moderate positiveness (+).

2. The gastric juice in cases of gastric and/or duodenal ulcer showed a slight tendency to increase in the number of occurring amino acids, but the degree of strong positiveness (++) occurred in only 1 case. The rate of occurrence after gastrectomy was nearly the same as before operation, but strong positiveness disappeared after gastrectomy.

3. In the gastric juice of gastric carcinoma cases, proline, threonine, and a peptide newly appeared in addition to the amino acids seen in ulcer cases; the degree of occurrence of leucine, valine, alanine, serine and glycine, and glutamic acid was remarkably high. The free amino acids in the remaining stomach after gastrectomy showed, in comparison with those before operation, a pronounced depression in the degree of occurrence; and their state of occurrence became similar to that seen in ulcer cases before operation.

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Precipitin Reaction of Gastric Juice of Patients with Gastric Cancer

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In 1904 Maragliano[1] published a procedure for diagnosing gastric cancer by precipitin tests between the gastric juice of patients with gastric cancer and the rabbit serum against a saline extract of human gastric cancer, and the method has recently been modified by Yamamoto[2] and Tomoda[3]. To clarify specific antigens responsible for the precipitin reaction, the present work was undertaken. Gastric juice was collected in great quantities from normals and from patients with gastric cancer and evaporated to powder. These crude materials were physically and chemically treated, and the effect of each treatment on the specificity of the substances as antigen was examined. Further, the materials were fractionated into mucoprotein and mucoproteose and the specific antigenicities of them were traced.

Experiments and Comments

Preparation of a Crude Material from Gastric Juice

Gastric juice was collected from patients with gastric cancer (hereafter referred to simply as cancer patients) by Katsch's method, and filtered. The filtrate was concentrated in vacuum at 37° to 39°C. to a syrupy state, to which was added 5 times as much absolute alcohol. The precipitate was dried in a vacuum desiccator to powder, which was dissolved in water and again dried to powder. The yield of this crude powder of gastric juice (to be referred to as **Cr. I**) was 100 to 120 mg. per 100 cc. of the gastric juice collected.

Similarly, a crude powder of " normal " gastric juice was prepared, for each blood group, from the gastric juice of patients with other surgical diseases than malignant tumor.

Preparation of Antisera

Rabbit sera against saline extracts of gastric cancer and normal gastric mucosa were the same as those used by Kobayashi[4].

Tests for the Presence of Specific Antigens in the Gastric Juice of Patients with Gastric Cancer

The precipitin reaction of anti-gastric cancer serum with the gastric juice of cancer patients gave the same results as the reaction with normal gastric juice, the titer being 1600 in both cases (Table I). When 4 drops

TABLE I

Precipitin Reaction of Crude Gastric Juice Powder with the Anti-gastric cancer Serum

Antiserum : Rabbit serum against the saline extract of human gastric cancer.
Antigen : A 1 :50 solution of a crude gastric juice power was doubly diluted.

1 cc. antiserum absorbed with	Antigen: gastric juice of	50	100	200	400	800	1600	3200	6400	Control antigen
(1) Normal human serum 4 drops	Cancer patient	+	+	+	+	+	−	−	−	−
(2) 1:50 solution of crude powder of normal human gastric juice 20 drops	Normals	+	+	+	+	+	−	−	−	
	Control antiserum									

of the normal human serum of the O group was added to 1 cc. of anti-gastric cancer serum to remove species-specific antibodies, precipitin tests which were made after 2 hours' incubation of the serum mixture at 37°C. yielded the results as shown in the left area of Table I (surrounded by heavy lines) that the titer was 400 in both cases. When 1 :50 solution of the crude powder of normal gastric juice was added to the above-mentioned anti-gastric cancer serum (absorbed with normal serum) at the rate of 2 drops per 0.1 cc., only the gastric juice powder from cancer patients showed a positive precipitin reaction with the antiserum, as shown in the upper left corner of the table (surrounded by broken lines), a result being indicative of the presence of specific antigens in the gastric juidce of cance patients.

Specific Antigenicities of the Gastric Juice and Gastric Mucosa of Normals and Cancer Patients

The crude powder of gastric cancer or of normal gastric mucosa was prepared as follows: An extract of fresh tissue with physiological saline

was dialyzed and concentrated, to which was added alcohol. The precipitate was dried to powder. The yield was 50 to 70 mg. per 10 g. of fresh tissue.

1) Tests of anti-gastric mucosa serum

a) Precipitin tests of anti-gastric mucosa serum absorbed first with normal serum, then with the gastric juice of cancer patients were positive with normal gastric juice, normal gastric mucosa, and gastric cancer (Table II A).

TABLE II

Preciptin Reaction of Crude Gastric Juice Power or a Crude Tissue Powder with the Anti-gastric mucosa Serum

Antiserum: Rabbit serum against the saline extract of normal human gastric mucosa. Antigen: A 1:50 solution of a crude gastric juice powder or a crude tissue powder was doubly diluted.

	1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1600	3200	6400	Control antigen
A	(1) Normal human serum 4 drops	Normal gastric juice	+	+	−	−	−	−	−	−	−
	(2) 1:50 solution of crude powder of gastric juice of cancer patient 20 drops	Gastric juice of cancer patients	−	−	−	−	−	−	−	−	
		Normal gastric mucosa	+	+	−	−	−	−	−	−	
		Gastric cancer	+	−	−	−	−	−	−	−	
B	(1) Normal human serum 4 drops	Normal gastric juice	+	+	−	−	−	−	−	−	
	(2) 1:50 solution of crude powder of human gastric cancer 20 drops	Gastric juice of cancer patients	−	−	−	−	−	−	−	−	
		Normal gastric mucosa	+	+	+	−	−	−	−	−	
		Gastric cancer	−	−	−	−	−	−	−	−	
		Control antiserum									

b) Tests of the antiserum absorbed first with normal serum, then with gastric cancer were positive only with normal gastric juice and normal gastric mucosa (Table II B).

2) Tests of anti-gastric cancer serum

a) Tests of anti-gastric cancer serum absorbed first with normal serum, then with normal gastric juice were positive with the gastric juice of cancer patients, gastric cancer, and normal gastric mucosa (Table III A).

b) Tests of the antiserum absorbed first with normal serum, then with normal gastric mucosa were positive only with the gastric juice of cancer patients and gastric cancer (Table III B).

TABLE III

Precipitin Reaction of Crude Gastric Juice Powder or Crude Tissue Powder with the Anti-gastric cancer Serum

Antiserum : Rabbit serum against the saline extract of human gastric cancer.
Antigen : A 1:50 solution of a crude gastric juice powder or a crude tissue powder was doubly diluted.

	1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1600	3200	6400	Control antigen
A	(1) Normal human serum 4 drops	Normal gastric juice	−	−	−	−	−	−	−	−	−
	(2) 1:50 solution of crude powder of normal human gastric juice 20 drops	Gastric juice of cancer patients	+	+	−	−	−	−	−	−	
		Normal gastric mucosa	+	+	+	+	+	−	−	−	
		Gastric cancer	+	+	+	−	−	−	−	−	
B	(1) Normal human serum 4 drops	Normal gastric juice	−	−	−	−	−	−	−	−	
	(2) 1:50 solution of crude powder normal gastric mucosa	Gastric juice of cancer patients	+	+	−	−	−	−	−	−	
		Normal gastric mucosa	−	−	−	−	−	−	−	−	
		Gastric cancer	+	+	−	−	−	−	−	−	
		Control antiserum									−

These results suggest that normal gastric juice has some specific antigens common with normal gastric mucosa, and the gastric juice of cancer patients has some specific antigens common with gastric cancer.

Effects of Physical and Chemical Treatments of the Cancer Patients' Gastric Juice on its Specific Antigenicity

The gastric juice of cancer patients was heated, frozen, or treated with acid, alkali, or formalin, and the effect of each treatments on the specific antigenicity was examined. The antiserum used was antigastric cancer serum that had been absorbed on full saturation first with normal serum, then with the crude powder of normal gastric juice.

1) Effect of heating. Three portions of a 1:50 solution of the crude powder from the gastric juice of cancer patients (Cr. I) were heated at 70°C. for 30 minutes, at 100°C. for 10 minutes, or at 100°C. for 20 minutes, with the results (as shown in Table IV A) that the specific antigenicity was decreased by 10 minutes' heating at 100°C., and lost by 20 minutes' heating at 100°C.

2) Effect of freezing. A 1:50 solution of Cr. I was frozen by means of dry ice, and after 10 minutes it was allowed to melt naturally. The

freezing and melting was repeated twice further. It was found that after these treatments the precipitin reaction became much weakened (Table IV A).

3) Effect of treating with a weak acid. An amount of 10 mg. of Cr. I was dissolved in 1.0 cc. of distilled water, to which was added 2 drops of 98% glacial acetic acid. After being left for 30 minutes, the mixture received 20 cc. of distilled water, and was concentrated in vacuum, to which was added again distilled water, and the mixture was concentrated in vacuum. This process was repeated until the mixture became neutral. The final mixture was concentrated to a syrupy state, to which was added absolute alcohol, and the precipitate was dried in a desiccator (the yield being 7.5 mg.). The precipitin tests with this dried material were negative (Table IV A).

4) Effect of treating with a strong alkali. An amount of 10 mg. of Cr. I was dissolved in 1.0 cc. of distilled water, to which was added 2 drops of 10% sodium hydroxide, and the mixture was left for 30 minutes. Absolute alcohol was then added, and the precipitate was repeatedly washed with alcohol until the washing became neutral. Finally, the precipitate was dried to powder (the yield being 8.5 mg.). This dried material gave negative precipitin tests (Table IV A).

5) Effect of treating with formalin. An amount of 10 mg. of Cr. I was dissolved in 1.0 cc. of distilled water, to which was added 0.05 cc. of 40% formalin, and the mixture was left for 2 hours. Absolute alcohol was then added; the precipitate was repeatedly washed with alcohol, and dried to powder (the yield being 7.4 mg.). Further, to see the extent of denaturation of the antigens by alcohol, control experiments were run in which the Cr. I solution was treated in the same manner as above but without addition of formalin. It was found that the precipitin reaction was only slightly weakened by the alcohol treatment, while the formalin treatment destroyed the specific antigens (Table IV A).

Fractionation of the Crude Gastric Juice Powder of Cancer Patients

The crude powder of the gastric juice of cancer patients was fractionated with ammonium sulfate or dioxane, and the specific antigenicity of gastric cancer was traced in each fraction; the powder was fractionated also into mucoprotein and mucoproteose to examine the transference of the specific antigenicity. The antiserum used was anti-gastric cancer serum that had previously been absorbed on full saturation with normal serum and the crude powder of normal gastric juice.

I. Fractionation by means of ammonium sulfate

An amount of 150 mg. of Cr. I was dissolved in 4.0 cc. of distilled

TABLE IV

Effects of Various Physical and Chemical Treatments on the Precipitin Titer of Crude Gastric Juice Powder from Patients with Gastric Cancer

Antiserum: Rabbit serum against the saline extract of human gastric cancer.
Antigen: A 1:50 solution of a physically or chemically treated gastric juice powder of gastric cancer patients was doubly diluted.

	1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1600	3200	6400	Control antigen
A	(1) Normal human serum 4 drops	Heating 70°C., 30 min.	+	+	−	−	−	−	−	−	−
	(2) 1:50 solution of crude powder of normal human gastric juice 20 drops	100°C., 10 min.	+	−	−	−	−	−	−	−	
		100°C., 20 min.	−	−	−	−	−	−	−	−	
		Freezing	+	+	−	−	−	−	−	−	
		Acid	−	−	−	−	−	−	−	−	
		Alkali	−	−	−	−	−	−	−	−	
		Formalin	−	−	−	−	−	−	−	−	
		Alcohol	+	+	−	−	−	−	−	−	
		Not treated	+	+	+	−	−	−	−	−	
B	ditto	Dioxane Ppt. by 46%	+	+	+	+	−	−	−	−	
		Ppt. by 50%	−	−	−	−	−	−	−	−	
		Supernatant by 50%	−	−	−	−	−	−	−	−	
C	ditto	Ammonium sulfate Ppt. on half satu.	+	+	−	−	−	−	−	−	
		Ppt. on full satu.	+	+	−	−	−	−	−	−	
		Supernatant on full satu.	±	+	−	−	−	−	−	−	
		Control antiserum									−

water, and the insoluble part was discarded. To the supernatant was added an equal volume of saturated ammonium sulfate. Afte being kept for 30 minutes at 37°C., the mixture was centrifuged; the precipitate obtained was repeatedly washed with halfsaturated ammonium sulfate, dissolved in 20 cc. of distilled water, dialyzed through cellophane, and concentrated. Alcohol was added to the concentrate, and precipitate was dried (the yield being 5.5 mg.). To 15 cc of the supernatant halfsaturated with ammonium sulfate was added 5.7 g. of ammonium sulfate crystals to obtain a fully saturated solution. The solution was left at 37°C. for 30 minutes and the precipitate was washed with saturated ammonium sulfate 3 times, dialyzed, and dried to powder (the yield being 12.0 mg.). The supernatant of the above-mentioned fully saturated solution was dialyzed and dried to powder (the yield being 30 mg.).

Precipitin tests performed with these 3 fractions gave the results that gastric cancer-specific antigens were present in all of the 3 fractions (Table IV C).

II. Fractionation by means of dioxane

An amount of 2.0 g. of Cr. I was dissolved in distilled water, and the undissolved matter was removed. To the supernatant (18.9 cc.) was added 16.1 cc. of pure dioxane to give a final concentration of 46%. The mixture was left overnight in an ice chamber. Centrifuged. The supernatant was set aside, and the centrifugate was repeatedly washed with 46% dioxane, dried to powder, redissolved in water at pH 8.0. To the solution was added dioxane to give a final concentration of 46%. The resulting precipitate was dried to powder, and then redissolved at pH 8.0; acetone was added to the supernatant, and the precipitate was dried to powder (to be referred to as precipitate by 46% dioxane, the yield being 18.2 mg.).

To 40 cc. of the supernatant of the precipitate by 46% dioxane was added 3.2 cc. of pure dioxane to give a final concentration of 50%; the mixture was left overnight, and the resulting precipitate was repeatedly washed with 50% dioxane, and dried to powder (the yield being 5 mg.).

To the supernatant of the precipitate by 50% dioxane was added absolute alcohol, and the precipitate obtained was dried (the yield being 401 mg.).

On making precipitin tests with these 3 fractions it was found that only the precipitate with 46% dioxane was positive for gastric cancer-specific antigens (Table IV B).

III. Fractionation into mucoprotein and mucoproteose

Gastric juice was fractionated by the Glass and Boyd method.[5] To 6.5 g. of the curde powder of the gastric juice from the cancerous patients was added 30 cc. of distilled water and the pH was adjusted at 7.6 with *N*/100 sodium hydroxide. The mixture was centrifuged and the undissolved matter was discarded (1.05 g.). To 58.0 cc. of the supernatant was added 29.0 cc. of 10% trichloroacetic acid, and the mixture was left at room temperature for 15 minutes, and centrifuged for 25 minutes at 10000 rpm. The supernatant was set aside, and the precipitate was dried after being washed first with 3.3% trichloroacetic acid, then with acetone-water (3:1) (the yield being 250 mg.).

To the supernatant was added 1.5 times as much pure acetone, and the mixture was allowed to stand for 1 hour at 40°C.; the precipitate obtained was repeatedly washed with acetone, and dried to powder. To the powder was added 12 cc. of distilled water. After adjusting the pH at 8.0 with *N*/100 sodium hydroxide, the mixture was centrifuged and the insoluble part was rejected. Then the pH of the supernatant was lowered

to 3.7 by adding 10% hydrochloric acid; the resulting precipitate was repeatedly washed first with the hydrochloric acid solution at pH 3.7, then with acetone-water, and finally dried to powder (the yield being 70 mg.). The final substance obtained was mucoproteose as named by Glass and Boyd. To 20 cc. of the above supernatant of pH 3.7 was added pure acetone; the precipitate produced was separated by centrifugation, washed with acetone-water, and dried to powder (the yield being 450 mg.). The final substance obtained was so-called mucoprotein.

To 160 cc. of the supernatant obtained after the addition of acetone was added 800 cc. of absolute alcohol. The resulting precipitate was repeatedly washed with alcohol and dried to powder (the yield being 280 mg.).

TABLE V

Precipitin Reaction of Mucoprotein or Mucoproteose with the Anti-gastric cancer Serum

Antiserum: Rabbit serum against the saline extract of human gastric cancer.
Antigen: A 1:50 solution of mucoprotein or mucoproteose was doubly diluted.

1 cc. antiserum absorbed with	Antigen	50	100	200	400	800	1600	3200	6400	Control antigen
(1) Normal human serum 4 drops	Normal gastric juice Mucoprotein	+	−	−	−	−	−	−	−	−
	Normal gastric juice Mucoproteose	−	−	−	−	−	−	−	−	
(2) Normal gastric juice mucoprotein and mucoproteose, either 10 mg.	Gastric juice of cancer patients Mucoprotein	+	+	+	−	−	−	−	−	
	Gastric juice of cancer patients Mucoproteose	+	+	−	−	−	−	−	−	
	Control antiserum									−

Normal gastric juice was fractionated in the same manner as above.

Precipitin tests of anti-gastric cancer serum that had been absorbed first with normal serum, then with the mucoprotein and mucoproteose obtained from normal gastric juice were performed with the mucoprotein and mucoproteose of normal gastric juice and of the gastric juice of cancer patients. As shown in Table V, it was found that the mucroprotein and mucoproteose of the gastric juice of cancer patients gave positive tests.

SUMMARY

1. The presence of specific antigens in the gastric juice of patients

with gastric cancer has been demonstrated.

2. The gastric juice of the cancer patients has some specific antigens common with the gastric cancer tissue.

3. The specific antigens found in the gastric juice of the cancer patients were destroyed by heating, freezing, and treating with a weak acid, a strong alkali, and formalin.

4. Of various fractions obtained by the use of ammonium sulfate, the following three fractions were found to contain specific antigens: the precipitate obtained on half saturation, the supernatant obtained on full saturation, and the precipitate obtained on full saturation with ammonium sulfate.

5. Of various fractions obtained by the use of dioxane, only the precipitate produced by 46% dioxane showed specific antigenicity.

6. Each of the mucoprotein and the mucoproteose obtained from the gastric juice of the cancer patients showed specific antigenicity.

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A, B and O Group Mucopolysaccharides from Human Stomach Mucosae Are "Toxohormone" Active—Preliminary Note

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Human stomach mucosa from non-cancerous patients belonging to group A, B or O was extracted with water, and the solution was freed from the precipitate occurring on acidification to pH 4.5. The substance recovered from the centrifuged supernatant was fractioned at first by saturation of its solution with ammonium sulfate. The deposit thereby was dissolved in 1.9% barium acetate, and ethanol was added in portions. The fraction precipitating at the concentration range of ethanol from 40 to 60% was liberated from barium acetate, and after dissolution in 90% phenol, partially precipitated by addition of ethanol to 10%—$(NH_4)_2SO_4$-precipitable group mucopolysaccharide (GGS I). The above mother fluid saturated with ammonium sulfate was dialyzed, concentrated and precipitated with ethanol. The deposit here was subjected to fractionated precipitation with ethanol at first from 0.2 *M* acetate buffer, pH 4.6, I=0.2 and then from 0.93% barium acetate, taking in each case the despoits at ethanol concentrations 35–60%. The fraction given was dissolved in water, treated with Amberlite IR 120 and IRA 410, concentrated to a syrup and precipitated with ethanol containing some sodium acetate—$(NH_4)_2SO_4$-unprecipitable group mucopolysaccharide (GGS II). Of GGSs I group B mucopolysaccharide (GGS I-B) only has been isolated, and of GGSs II group A, B and O mucopolysaccharides (GGS II-A, II-B, II-O). All the preparations proved homogeneous electrophoretically as well as ultracentrifugally. GGS I-B: N 5.7%, hexosamine 29.4%, ash 0.4%, $[\alpha]_D^{\infty}$ (water) $=-7.1°$; GGS II-A: N 5.0%, hexosamine 33.5%, ash 1.0%, $[\alpha]_D^{18}$(water) $=-19.6°$; GGS II-B: N 4.7%, hexosamine 32.3%, ash 0.4%, $[\alpha]_D^{18}$ (water) $=-28.2°$; GGS II-O: N 5.0%; hexosamine 34.3%, ash 0.6%, $[\alpha]_D^{17}$ (water) $=-34.5°$. All were shown to distinctly depress the liver catalase activity as determined gasometrically, when their 5 mg. amounts were injected into mice intraperitoneally. Blood- and kidney-catalase were not affected in vivo nor liver catalase in vitro. They have no KIK-potency. GGS I-A and I-O are analogized to be similar in respects with the toxohormone and KIK actions.

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Biochemical Studies on Carbohydrates

CCVIII. Oligosaccharides Separated after Acetolysis of the Group Mucopolysaccharide from Pig Gastric Mucus Third Report: "Gastro-N-disaccharide"

By

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Masamune and others[1),2)] reported before on "gastro-N-trisaccharide" and acetyllactosamine as deacetylated acetolysis products of the group mucopolysaccharide from pig stomach mucus. This account deals with another crystalline product composed of equimolecular galactose and acetylgalactosamine. It used alkaline iodine as much as 47.6% as expressed as glucose and gave galactonic acid on hydrolysis of the oxidation product. It consumed 3.9 moles of sodium metaperiodate and gave 2.0 moles of formic acid and 1.4 moles of formaldehyde per mole. Red coloration occurred when it was tested with alkaline triphenyltetrazolium chloride reagent, but Osaki-Turumi (Morgan-Elson) reaction resulted in negative. It rotated D-light remarkably to the right and absorbed strongly infrared rays at and near 824 cm.$^{-1}$, but did not at all at 895±9 cm.$^{-1}$.[3)] These findings establish the structure of galactose-4-α-N-acetylgalactosaminide. This disaccharide will be named "gastro-N-disaccharide".

Experimental

Material

The group mucopolysaccharide was prepared by a modification of Yosizawa procedure[4)] as follows:—

First stage. 500 g. of pig stomach mucus was taken up in 1.5 *N* NaOH and stood at 0–5°C for 24 hours. The solution given was acidified to pH 5.0 with glacial acetic acid, and after agitation with 300 g. of Celite, filtered under suction. The bulk on the funnel was washed with dilute acetic acid of pH 5.0. The filtrate and washing were dialyzed in cellophane bags against running water until neutral. Some precipitate was removed on a folded filter, and the filtrate here was distilled in vacuo to about 80 cc., followed by addition of 5.5 volumes of 95% ethanol. The deposit was washed with 95% ethanol and stood in vacuo over anhydrous calcium chloride. Yield at this stage 13.0 g.

Second stage. It was kneaded with a small volume of water and made up to 40 cc. with water. After rejection of the insoluble part, the solution was adjusted to pH 4.5 with dilute acetic acid and centrifuged, the centrifugate was dissolved in a few cc. of water, acidulated and centrifuged, and the supernatant fluids combined were distilled in vacuo to about 20 cc.

Third stage. To the condensate were added 15 volumes of glacial acetic acid, and the mixture was stood at room temperature for 24 hours, stirring occasionally. The deposit was centrifuged off, washed with 30 cc. of glacial acetic acid and with 30 cc. of 95% ethanol in turn and placed in a vacuum desiccator. 4.5 g. of a grayish powder was given.

Fourth stage. It was dissolved in 450 cc. of water, 90 cc. of 12.5% $CuSO_4$ was added, and the mixture was further added to with 60 cc. of 10% NaOH in small portions. The precipitate was washed twice with 1% NaOH and dissolved by addition of 6 *N* HCl to pH 2.0 under cooling on ice, followed by making up to 450 cc. with water. The solution was neutralized with 10% NaOH under cooling, 50 cc. of 10% NaOH was further added, and the deposit was washed with 1% NaOH and dissolved with 6 *N* HCl similar to above. And this solution was precipitated with 7 volumes of abs. ethanol, centrifuged off, washed with three changes of a mixture of concentrated hydrochloric acid and abs. ethanol (pH 2.0) and two changes of abs. ethanol in succession and dried.

The procedure above was repeated twice more. 3.5 g. of a grayish powder was given.

Fifth stage. It was dissolved in 175 cc. of water, passed through a 2.5×30 cm. column of Amberlite IR 120 (H form) and washed with 50 cc. of water. The effluent and washing were neutralized with 1 *N* NaOH, distilled in vacuo to about 15 cc. and dried after precipitation with 10 volumes of abs. ethanol. Crop 3.3 g.

The whole procedure of preparation was repeated with many batches of the mucus.

Isolation of the Disaccharide

1) Acetolysis and deacetylation of the group mucopolysaccharide. The procedure of Masamune *et al.*[1] was applied.

2) Fractionation was carried out according to Whistler and Durso[5]. 20 g. of the deacetylated product was taken up in 200 cc. of water and added to a 5×48 cm. column of a mixture of charcoal and Celite in a weight proportion of 1:1, and 16 *l.* of water and 20 *l.* of 2.5% (v/v) ethanol were added in drops successively.

The effluent after addition of water was discarded and that after addition of 2.5% ethanol was collected in 2 *l.* fractions. The fractions were separately distilled in vacuo to dryness. The still-residues were found to contain a contaminant that forms floccules in water so that they were taken up each in 5 cc. of water, filtered through a Toyo Roshi filter paper No. 85 fitted in a Seitz filter and washed with three 2 cc. portions of water. The filtrate and washing

were distilled in vacuo to dryness. Since, however, the still-residues thus given were not as yet free from the insoluble contaminant, they were subjected to the same manipulations five times more. The clear-soluble still-residues were washed separately with 5 cc. and 3 cc. portions of ice-chilled abs. methanol, dissolved in 2 cc. of water, and after addition of a few drops of butanol until appearance of slight cloudiness, stood for 24 hours at room temperature. Crystals in long thin needles appeared, which were filtered off on a suction funnel, washed with 5 cc. of methanol chilled on ice and 5 cc. of ether and dried in air. Yielded were 30, 20 and 15 mg. from the first, second and third effluent fractions respectively. Subsequent fractions gave none of the product. F. P. 186–187°C (decomp.). The melting point was not elevated, when the product was recrystallized from abs. methanol.

Properties of the Product

Test tube tests. Molisch positive; Osaki-Turumi negative.

Paper partition chromatography. Chromatograms were developed on slivers of Toyo Roshi filter paper No. 3 at 25°C by descending irrigation with various solvent mixtures and indicated with the aniline hydrogen phthalate reagent of Partridge or T.T.C. reagent of Wallenfels.[6] The Rf values calculated are embodied in Table I.

TABLE I

$R_{glucose}$ and $R_{lactose}$ Values of Gastro-N-disaccharide

Solvent mixture	$R_{glucose}$	$R_{lactose}$
Butanol-pyridine-water (5:3:2 by volume)[7]	0.63	1.39
Butanol-acetic acid-water (4:1:5 by volume, 25°C, upper layer)[8]	0.83	1.61
Isopentanol-butanol-water (7:1:2 by volume, 25°C, upper layer)[9]	0.75	1.50
Phenol satd. w. water (25°C)[10]	1.58	1.58

Acid hydrolysis. 5 mg. of the substance was sealed in a tube with 1 cc. of 1 *N* HCl and heated at 100°C for 5 hours. The hydrolysate was freed from the chloride ions by passing through a 1.5×3 cm. column of Amberlite IR 45 (OH form) and washed with 10 cc. of water. The effluent and washing were next passed through a column of the same size as above of Amberlite IR 120 (H form) and washed with 10 cc. of water. The effluent and washing here were distilled in vacuo, the dry still-residue was dissolved in 0.1 cc. of water, and 0.05 cc. of the solution was paper-chromatographed descendingly with Solvent butanol-pyridine-water (5:3:2 by volume) of Masamune and Yosizawa[7] on Toyo Roshi filter paper No. 3. Temperature and duration of irrigation were 25°C and 15 hours respectively. Indicator aniline hydrogen phthalate reagent of Partridge. One spot only was given at the position corresponding to galactose.

The column of Amberlite IR 120 used above was washed with 10 cc. of 0.5 *N* HCl. The eluate was distilled in vacuo at 35°C to about 1 cc., and after addition of 10 cc. of abs. methanol, again distilled in a similar manner. The

distillation with methanol was repeated four times, until the hydrochloric acid was mostly expelled, and the final condensate was distilled to dryness. The still-residue was taken up in 0.1 cc. of water, and 0.05 cc. of the solution was paper-chromatographed descendingly (Irrigation solvent the mixture of butanol, pyridine and a dilute hydrochloric acid of Masamune and Yosizawa[11]; paper slivers Toyo Roshi filter paper No. 3; temperature 25°C; duration of irrigation 40 hours; indicator aniline hydrogen phthalate reagent of Partridge). Galactosamine hydrochloride was detected but no glucosamine hydrochloride.

Quantitative analyses. Decrease of weight did not occur when it was heated at 105°C in vacuo over P_2O_5 for 5 hours. Analysis revealed it to be a disaccharide composed of N-acetylgalactosamine and galactose. See Table II.

TABLE II

Composition of Gastro-N-disaccharide

Analysis	Found (%)	Calcd. for $C_{14}H_{25}O_{11}N$ (383.3)
N*	3.7	3.65
Galactose†	47.2	47.0
Galactosamine‡	46.1	46.7
Acetyl§	10.4	11.2
Iodine use as glucose//	47.6	47.0

* Micro Kjeldahl. † Method HR_{10} of Masamune and Sakamoto.[12] ‡ Masamune and Yosizawa.[13] § Suzuki.[14] // Macleod and Robison.[15]

Optical rotation in water.

$$[\alpha]_D^{17} = \frac{+2.24° \times 100}{1.05 \times 1} = +213.7° \text{ (5 min.)}$$

$$\longrightarrow \frac{+2.13° \times 100}{1.05 \times 1} = +203.2° \text{ (24 hrs.)}$$

Infrared spectrum (The writer is indebted to Mr. Y. Ikegami in the Chemical Institute of Non-aqueous Solutions). The infrared spectrum was recorded in a Perkin-Elmer Model 21 recording infrared spectrophotometer with a NaCl prism, using the KBr disk method. See Fig. 1.

Oxidation with hypoiodite and examination of the oxidation product. 10 mg. of the substance was dissolved in 3 cc. of water, 12 cc. of 0.02 *N* iodine and 0.6 cc. of 0.5 *N* Na_2CO_3 were added, and the mixture was stood in a water-bath at 21°C for 45 minutes, avoiding light. The reaction mixture was then agitated with 3 g. of Dowex 50 (H form), filtered and washed with three 3 cc. portions of water. The filtrate and washing were shaken vigorously with seven 30 cc. portions of chloroform, until the yellow color of iodine was removed, and distilled in vacuo. Since the dry still-residue was still tinged yellow, it was washed with ether twice. The perfectly colorless substance weighing 8.1 mg. was dissolved in 5 cc. of water for acid hydrolysis with following paper chromatography (a) and quantitative analyses (b).

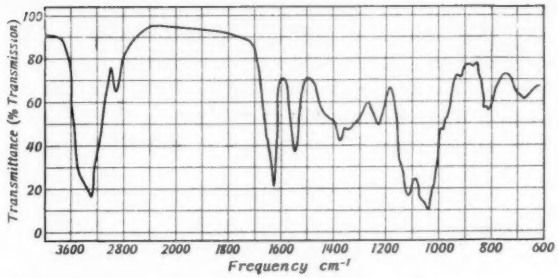

Fig. 1. Infrared spectrum of gastro-N-disaccharide.

a) 2 cc. of the solution was sealed with an equal volume of 2 *N* HCl in a tube and heated at 100°C for 5 hours. The hydrolysate was evaporated in vacuo over anhydrous calcium chloride and solid caustic soda at 35°C to a small volume. It was repeatedly evaporated together with 1 cc. of water to eliminate the hydrochloric acid and finally dried. The residue was dissolved in 0.12 cc. of water, and three 0.03 cc. portions of the solution were sampled on separate slivers of Toyo Roshi filter paper No. 3 to descendingly chromatograph with Solvent butanol-pyridine-water (5:3:2 by volume). Temperature and duration of irrigation were 25°C and 14 hours respectively. The chromatograms were indicated with Partridge aniline hydrogen phthalate reagent, B.P.B. reagent (Dissolve 40 mg. of B.P.B. in 100 cc. of 99% ethanol and add drops of 1 *N* NaOH just sufficient to turn blue the solution) or Abdel-Akhel and Smith's lactone reagent.[16] One spot corresponding to galactosamine, two spots corresponding to galactonic acid and its lactone (The lactone was indicated by the B.P.B. reagent probably due to the opening of the lactone ring by the excess alkali in this reagent) and one spot corresponding to galactonolactone were shown by the indicators in the order arranged above respectively. For the reference runs were used galactosamine hydrochloride, galactose, galactosaminic acid and a mixture of galactonic acid and its lactone, of which the last two were prepared as follows.

Galactosaminic acid: It was prepared according to Wolfrom and Cron.[17]

Mixture of galactonic acid and its lactone: Galactose was oxidized with hypoiodite as described above regarding the disaccharide in question, and after adsorption on an Amberlite IR 45 (OH form) column, desorbed with 0.5 *N* HCl. The eluate was condensed, freed from the hydrochloric acid by distilling with methanol to a small volume repeatedly and finally distilled to dryness. Yielded was 0.7 g. from 1 g. of galactose.

b) Analytical figures below were obtained.

Analysis	Found (%)	Calcd. for $C_{14}H_{25}O_{12}N$
Galactose*	0	0
Galactosamine†	48.0	45.0

* Method PHR_2 of Masamune and Sakamoto.[12] † Masamune and Yosizawa.[13]

Oxidation with sodium metaperiodate and analysis of the oxidant remaining unused

and oxidation products. 13.6 mg. of gastro-N-disaccharide, 13.8 mg. of anhydrous lactose and 14.4 mg. of anhydrous melibiose were dissolved separately in 10 cc. of 0.1 *M* acetate buffer of pH 4.2, and after addition of 1 cc. of 0.3 *M* $NaIO_4$, placed in a water-bath at 20°C, avoiding light. 1 cc. portions of the reaction mixtures were pipetted out at intervals to analyze on the oxidant, formic acid and formaldehyde as described by Masamune *et al.*[1] See Table III.

TABLE III

Oxidation of Gastro-N-disaccharide with $NaIO_4$

Time of oxidation (hrs.)	$NaIO_4$ consumption (mole/mole)			HCOOH production (mole/mole)			HCHO production (mole/mole)		
	Gastro-N-disaccharide	Lactose	Melibiose	Gastro-N-disaccharide	Lactose	Melibiose	Gastro-N-disaccharide	Lactose	Melibiose
5	2.5	3.2	5.5						
24	3.1	3.4	5.7	1.6	2.3	3.9	0.9		
48	3.8	4.5	5.8						
72	3.9	4.8	5.8	2.0	2.9	4.8	1.4	1.5	0

Discussion

Since its hypoiodite-oxidation product gave galactosamine and galactonic acid on acid hydrolysis, gastro-N-disaccharide has the galactose residue at its reducing terminal. The two monosaccharide components are not bound through 1–2 linkage, because the test with alkaline triphenyltetrazolium chloride resulted in positive (Cf. Feingold *et al.*[18], Barker *et al.*[19] and Haq and Whelan[20]). 1–3 and 1–6 linkages are excluded for the negative Morgan-Elson reaction (Cf. Kuhn *et al.*[21]) and the result of periodate oxidation. The disaccharide is extremely dextrorotatory ($[\alpha]_D = +214° \rightarrow +203°$) and absorbs infrared rays with an absorption maximum at 824 $cm.^{-1}$ but without any maximum at 895 ± 9 $cm.^{-1}$, suggesting an α-galactopyranoside. In short, the disaccharide in question is galactose-4-α-N-acetylgalactosaminide.

Recently Côté and Morgan[22] isolated D-galactose-3-α-N-acetyl-D-galactosaminide from a partial hydrolysate of the group A substance from an ovarian cyst fluid. Since this disaccharide is described to be Morgan-Elson positive and less dextrorotatory ($[\alpha]_D$ approximately $+150°$), it differs from gastro-N-disaccharide.

Summary

1. A disaccharide "gastro-N-disaccharide" was isolated as a deacetylated acetolysis product of the group mucopolysaccharide from pig

stomach mucus.

2. Its physical and chemical properties were examined.

3. It has been established to have the structure of galactose-4-α-N-acetylgalactosaminide by analysis, oxidation experiments with hypoiodite and periodate, Osaki-Turumi (Morgan-Elson) test and examination of its infrared spectral absorption.

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Pressor Action of Noradrenaline in Young Dogs

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The relative noradrenaline content of the adrenal gland in young animals was larger than that in adult ones.[1] Therefore, it is of interest to know whether the relative activities of noradrenaline to adrenaline in young animals differ from those in adult ones. In this view, the pressor action of noradrenaline was compared with that of adrenaline in young dogs in the present study.

One female and 4 male dogs, aged 10–16 days and weighing 530–750g., were experimented on in a supine position without general anesthesia. The carotid artery and the femoral vein were exposed under the local anesthesia with 1% procaine solution. A mercury manometer was connected with the artery in order to record the blood pressure and a small cannula was inserted in the vein for injection.

From the standard stock solution 0.1–3.0 μg. of DL-noradrenaline (Sankyo) or 0.25–4.0 μg. of L-adrenaline (Sankyo) were taken and diluted up to 1.0 ml. with 0.9% NaCl solution. Each solution was injected through the cannula in 10 seconds.

As for the pressor action, DL-noradrenaline was more active than L-adrenaline in all cases. The activity ratio of DL-noradrenaline to L-adrenaline was estimated as 1.3 : 1–4 : 1, with the mean value of 2 : 1. Taking L-noradrenaline to be twice as active as DL-noradrenaline, the activity ratio of L-noradrenaline to L-adrenaline will be 4 : 1. Comparison of this figure with that in non-anesthetized adult dogs (1.6 : 1–2.0 : 1)[2] may lead us to the conclusion that the relative pressor action of noradrenaline in young dogs is definitely stronger than that in adult ones.

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Propagation Velocity and Total Reflection of Spreading Induction in the Light-Adapted Human Retina

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Introduction

A spreading process called " spreading induction " is started at the margin of an illuminated part of the retina, and, mediated by this process, a field of retinal induction is established around the illuminated area.[1-2] The velocity of propagation in the dark-adapted retina has been measured by three different methods[3-5], and consistent results have been obtained by these different methods. However, measurement of the velocity at the light-adapted retina seemed to be more difficult, because it was shown that spreading induction suffered from a stronger decrement in the light-adapted retina than in the dark-adapted.

Katayama and Aizawa[4] succeeded in measuring the velocity of spreading induction in the dark-adapted retina ; they measured the difference in time necessary for arrival of spreading induction at two points differently distant from the margin of an illuminated area of the retina. The velocity was given by the ratio of the distance between the two points to the difference in time measured.

In the present investigation, the same method was used to investigate the dependence of the velocity on the intensity of adapting light. On the other hand, a new indirect method which is based on the principle of total reflection has been introduced, and the data obtained by the direct and the indirect method were compared with each other.

Experimental

Method

The optical systems used are illustrated in Fig. 1. The lamp, L, of a slide-projector was used as a light-source. The intensity and the wave-length of light were controlled by means of suitable filters represented by G in Fig. 1. An

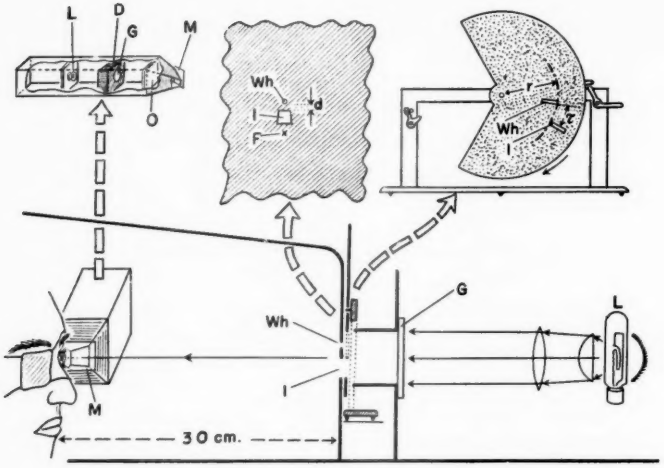

Fig. 1. Optical arrangements. D diaphragm, d distance between I and Wh, F fixation point, G glassfilter, I inducing light, L electric lamp, M half-mirror, O opaque glass, r radius of sector of photopendulum, Wh white test light. Further details in text.

inducing patch, I, and a white test patch, Wh, were presented on a vertical screen at a distance of 30 cm. in front of the subject's eye. The patch, I, was square in shape and 1×1 cm^2 in size. The test patch, Wh, was a circle of 1 mm. in diameter. The positions of I and Wh are shown in the inset. The distance, d, between I and Wh was variable. The eye was exposed to the two patches, I and Wh, successively by means of a sector pendulum, which is shown also in the inset. The exposure times of I and Wh were 2.5 msec., and the interval, τ, between I and Wh was variable.

Another set of optical system was used for light adaptation of the eye. The set consisted of a light-source, L, a diaphragm, D, smoked glass, G, and opaque glass, O, and a half-mirror, M. The latter was situated immediately before the eye so that the eye was subjected to a wide, uniform illumination. The intensity of the adapting light was controlled by D and G.

Curves showing retinal induction were obtained by these arrangements, and are illustrated in Fig. 2. In these experiments the intensity of the adapting light was 1 lux, and those of I and Wh 10,000 lux. The broken curve in this figure shows the change of electrical excitability of the eye following its exposure to the white test light alone. The continuous curves in the same figure refer to similar changes of electrical excitability following successive exposure to I and Wh, the interval between both exposures being 100 msec. The distance between I and Wh was 2.5 mm. on the vertical plane. The maximum of the continuous curve is seen at 2, 1, 2 or 3 sec. from the test flash, Wh, when the inducing flash was white, red, green or blue. The higher electrical excitability represented by

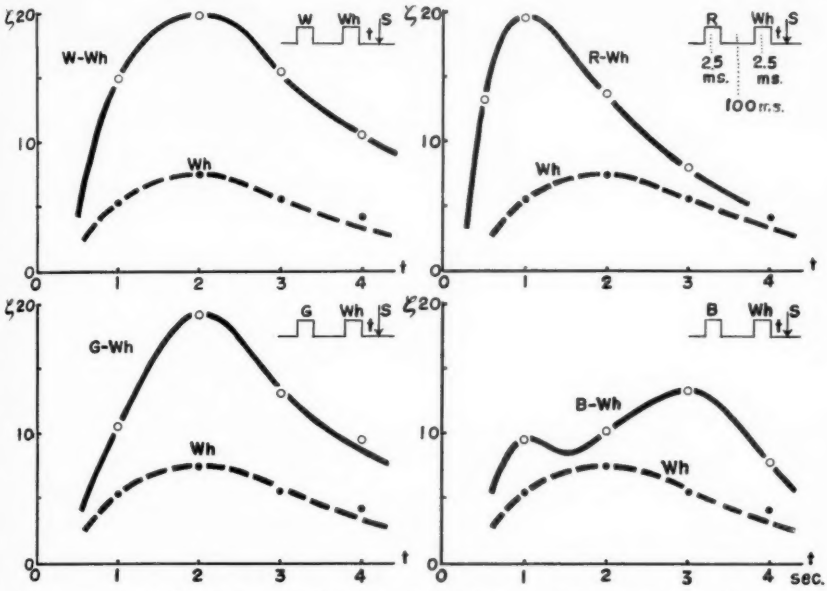

Fig. 2. Curves of retinal induction. Ordinates: Percentage increases in electrical excitability of eye as measured by a single rectangular shock of 0.1 sec. Abscissae: Time in seconds after white test light. Broken curves represent excitability curves obtained for white test light alone. Continuous curves represent curves of induction. Temporal sequence for procedure to obtain curves of induction is shown in inst. where W, R, G and B denote white, red, green and blue inducing lights. Wh and S stand for white test light and electric shock.

the continuous curve over the broken curve is a manifestation of retinal induction caused by the inducing light, I. The intensity of induction is measured by the maximal difference between both curves, and this difference is designated "contrast effect (C.E.)". The C.E. is generally a function of distance; it decreases exponentially as the distance increases, when the interval separating both exposures is fixed. With a fixed distance the C.E. depends on the interval between I and Wh. The dependence is shown in Fig. 3. In this figure, the C.E. is represented as a function of the interval, τ, for a given distance—say 2.5 mm. For this distance, the C.E. is zero when the interval is less than 5 msec. This means that no induction appears yet at this point when the test of induction is carried out too early with Wh. After a latency of about 5 msec. the C.E. increases almost linearly with the time interval and reaches a constant level, which is the higher the shorter the distance. It is also to be noted that the latency increases with the distance, and this fact may be so interpreted that the time necessary for propagation of spreading induction is included in the latency. If the velocity is constant, there must be a linear relation between the latency and the distance.

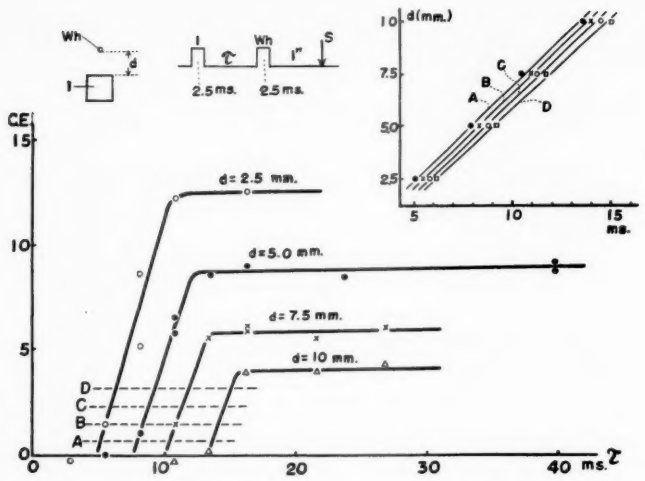

Fig. 3. Direct method for measuring velocity of spreading induction. Ordinates: Contrast effects. Abscissae: Time interval, τ, between red inducing light, I, and white test light, Wh. Arrangement of patches and experimental procedure are shown in inset, where d denotes distance between I and Wh. Latencies of C.E. were measured from intersections between horizontal lines A, B, C and D and C.E.-τ relations, and plotted aoainst distances, d, in inset.

In the inset of Fig. 3, distances are plotted as ordinates against latencies as abscissae. The latencies were determined from the intersections between the C.E. $-\tau$ relations and a horizontal line A, B, C or D. As can be seen in the inset, there is a linear relationship between the latencies and distances, regardless of the indicator used for determination of latencies. As the relations A, B, C and D are all parallel to one another, values of velocities determined by the slopes of these lines will be identical. The values so determined refer to the vertical plane 30 cm. in front of the eye. In order to obtain velocities in the retina, one has only to multiply these values by a factor 17/300, where the posterior nodal distance of the eye is assumed to be 17 mm.

Results

1) *Dependence of the velocity on the intensity of white adapting light*

By the method described above the dependence of the velocity on the adaptation level of the eye was investigated in two subjects. The results obtained are summarized in Table I. The intensity of the red inducing light used was 64,000 lux throughout this experiment. As can be seen in this table, the velocity increases with the intensity of the adapting light, and reaches a constant level at about 1 lux. It is to be noted that the velocity at the highest level is

TABLE I

Dependence of the Velocity of Spreading Induction on the Intensity of White Adapting Light

The Intensity of Red Inducing Light was 64,000 lux.

Intensity of adapting light in lux	Velocity in retina (mm.per sec.)	Subjects
0.000	1.7	K. M., T. K.
0.006	2.6	T. K.
0.010	4.2	K. M.
0.014	5.8	T. K.
0.040	16.7	K. M.
0.100	33.4	K. M.
0.400	39.0	K. M.
1.000	41.3	T. K.
4.000	42.8	K. M.

about 25 times as high as the velocity in the dark-adapted retina. In contrast to the remarkable effect of the intensity of adapting light, the intensity of inducing light has almost no effect upon the velocity, as will be shown in the following: The experiments were carried out at two different adaptation levels, and the intensity of the red inducing light was varied from 100,000 to 100 lux. As can be seen in Table II, the velocity is almost independent of the intensity of inducing light. In other words, the all-or-none law applies to the velocity of spreading induction.

TABLE II

The Intensity of Inducing Light (Red) and the Propagation Velocity of Spreading Induction in the Human Retina Adapted to White Light of Constant Intensity

Intensity of inducing light in lux	Velocity in retina (mm. per sec.)	
	Subject: K.M. Adapted to 4 lux	Subject: T.K. Adapted to 1 lux
100,000	37	39
10,000	36	44
1,000	41	37
100	38	–

Motokawa *et al.*[3] showed in a preceding paper that the velocity is independent of the wave-length of inducing light in the dark-adapted human retina. The same is true of the light-adapted retina; in the retina adapted to white light of 1 lux, values of velocity 38, 42, 39 and 41 mm./sec. were obtained in the

present experiment for red, green, blue and white inducing lights respectively. All these values may be regarded as identical with one another within the scope of experimental error.

2) *Total reflection of spreading induction*

The law of refraction may be represented in terms of velocities of light in two media as follows:

$$\frac{\sin i}{\sin r}=\frac{V_1}{V_2} \qquad (1),$$

where i, r, V_1 and V_2 denote the angle of incidence, the angle of refraction, the velocity in the first medium and that in the second medium respectively.

When V_2 is greater than V_1, a beam of light coming from the first medium is totally reflected at the boundary of the two media for angles of incidence greater than a certain critical value, i_c. In the special case in which the angle of incidence is equal to i_c, the refracted light runs parallel with the boundary surface. Then the following relation holds:

$$\frac{\sin i}{\sin r}=\frac{\sin i_c}{\sin 90^\circ}=\sin i_c=\frac{V_1}{V_2} \qquad (2).$$

If the critical angle, i_c, can be determined experimentally, the velocity in the second medium, V_2, may be determined from the known velocity, V_1, according to the formula (2).

As the velocity of spreading induction is greater in the light-adapted part of the retina than in the dark-adapted, the spreading induction coming from a dark-adapted part can totally be reflected at the margin of a retinal image of a white figure or of its after-image, because the retinal image or the after-image represents a light-adapted part of the retina. The phenomenon of total reflection can be demonstrated by the arrangement of patches shown in the inset of Fig. 4. In this arrangement I represents a blue inducing patch of 20 lux, P a white parabolic reflector to reflect the spreading induction caused by I, and Sc a white screen to prevent the spreading induction from reaching directly a linear white reflector, R, whose intensity was 0.04 lux. The reflector, R, was usually perpendicular to the axis of the parabolic reflector, and this position was regarded as standard. The parallel beams of spreading induction produced by the set of I and P will reach the linear reflector and be reflected there, if the angle of incidence is greater than the critical angle. The angle of incidence could be controlled by tilting the linear reflector from its initial position. D was a blue detector whose direct induction blue in character was to be neutralized by the yellow spreading induction initiated by the blue inducer, I. Wh was a white test light to test the state of induction at the detector, D. The patch, Wh, was always presented at the tip of the linear reflector. If the incident angle is critical, the refracted yellow induction will proceed along the upper margin of the linear reflector and reach the very part of the detector at which the neutralization is tested by Wh. Thus, neutralization will or will not be seen at this point according to whether the incident angle is critical or not.

The temporal sequence of experimental procedure is shown also in the inset

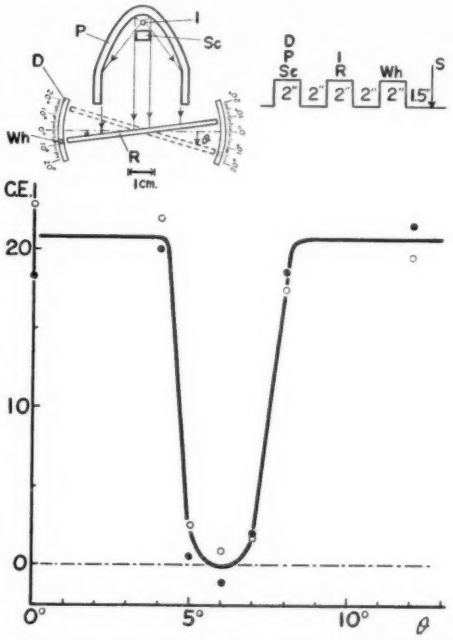

Fig. 4. Experiment to measure critical angle of incidence for total reflection of spreading induction. Arrangement of patches and experimental procedure are shown in insets. D : Blue detector of 20 lux. I : Blue inducer of 20 lux. P : White parabolic reflector. R : Linear reflector consisting of white line of 0.04 lux. R was tilted by angle from standard horizontal position. θ : Angle of incidence. Sc : White screen to prevent spreading induction from reaching directly linear reflector, R. Ordinates : Contrast effect at point Wh of detector. Abscissae : Angle of incidence. Neutralization of induction at point Wh occurs only when the refracted spreading induction proceeds along R.

of Fig. 4. We used two similar detectors symmetrically arranged on either side of the linear reflector so as to carry out similar experiments at symmetrical inclinations of the linear reflector and thus to eliminate the error due to a possible constructive defect of the patches used. The result is represented in Fig. 4, where the C.E.s at the detector are plotted as ordinates against the angles of incidence as abscissae. The data expressed by solid circles refer to the experiment carried out with the detector on the left side, and those expressed by empty circles to the experiment carried out with the right detector. As can be seen in this figure, the C.E. is zero over a range of incident angles from 5° to 7°. The critical angle must lie between these two extremes. For the time being, the middle value 6° will be adopted as the critical angle.

From formula (2) the velocity, V, in the retinal part, R, which was adapted to an illumination of 0.04 lux, will be given as follows :

$$V=\frac{\text{Velocity in dark-adapted retina}}{\sin\ (\text{critical angle})}=\frac{1.7}{\sin 6^\circ}=16.2\ \text{mm./sec.}$$

In the same way, velocities for various degrees of light adaptation were determined, the illumination of R being varied from 0.04 to 1 lux. The results are summarized in Fig. 5 I, in which A, B, and C refer to intensities of adapting light 0.04, 0.1 and 1 lux respectively. As can be seen in this figure, the range of incident angles over which the neutralization occurred was displaced from right to left as the intensity of the adapting light was increased. This means decreases in critical angle or increases in velocity with increasing intensities of adapting light.

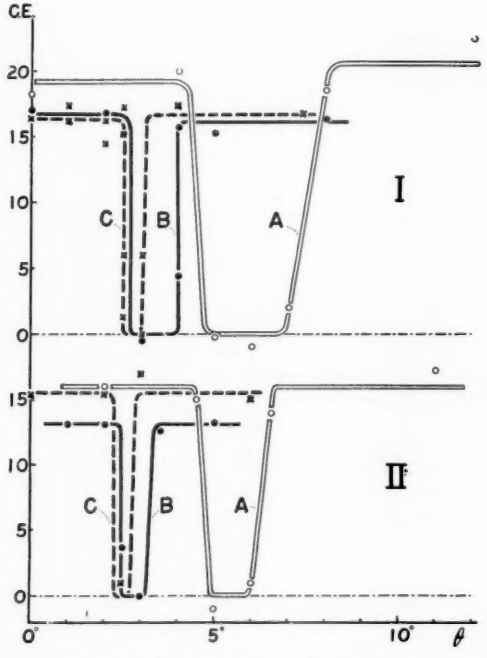

Fig. 5. Dependence of critical angle of incidence for total reflection of spreading induction on intensity of linear reflector, R. Intensity of R was 0.04, 0.1 and 1 lux for A, B and C respectively. Series I and II refer to intermittent and continuous illuminations of R respectively. Ordinates: Contrast effects at point Wh of detector, D (see inset of Fig. 4). Abscissae: Angle of incidence.

The velocity for each adaptation state was calculated in the same manner as stated above, and is represented in comparison with the corresponding velocity determined by the direct method in Table III. As can be seen in this table, the values for 0.04 lux by the direct and by the indirect method coincide with each

TABLE III

Dependence of the Velocity of Spreading Induction on the Adaptation State of the Eye

Intensity of adapting light in lux	Velocity in mm. per sec. (direct method)	Velocity in mm. per sec. (indirect method)	
		Intermittent adaptation	Continuous adaptation
0.04	16.7	16.7	17.9
0.1	33.4	28.7	34.2
1.0	41.3	35.4	39.0

other, but some discrepancy is seen between the data obtained by both methods for higher intensities; the values by the indirect method are somewhat lower than the corresponding ones by the direct method. What is responsible for this discrepancy? It seems that the difference of adapting procedure in the two methods is responsible for the discrepancy, because the latter was reduced by reducing the difference in experimental condition as far as possible in the further experiment to be mentioned. In the direct method the eye was exposed continuously to the adapting light of constant intensity, but in the indirect the linear reflector was presented only for a period of 2 seconds each time, and such procedure was repeated every 15 seconds. In another word, the light adaptation in the indirect method was intermittent, instead of continuous. Therefore, the initial part of the spreading induction reflected by the parabolic reflector must have passed through the linear reflector during illumination, but the later one which was traveling through the dark area between I and R just before the end of illumination must have passed through the after-image of R. It is obvious that the area of the after-image is less light-adapted than the area of the retinal image itself. This must be the reason why the values of velocity determined by the indirect method were lower than the corresponding ones by the direct method. We became aware of this situation after the experiment, and were compelled to carry out another series of experiments with a continuous illumination of the linear reflector, inspite of great difficulty in assuring sufficiently long fixation of the eye. The data obtained by this method are represented in Fig. 5 II. The curves A, B and C correspond to those marked by the same alphabets obtained by intermittent light adaptation in Fig. 5 I. It is to be noted that the range of neutralization is narrower in experiment II than in experiment I, probably because of more uniform light adaptation in II. The velocities computed from the data of experiment II are represented in the last column of Table III. As can be seen in this table, the agreement with the values obtained by the direct method has been improved by the continuous adaptation in the second series of experiment.

DISCUSSION

The values of velocity computed from the law of refraction have been

shown to agree satisfactorily with those determined by the direct method. The refracting medium used in our experiments was a retinal image of a white line. When the intensity of illumination of the line is over 1 lux, the critical angle for total reflection is only about 2.5°. Therefore spreading induction incident upon the retinal image of a white line with angle of incidence from 2.5° to 90° must be reflected by the retinal image. This means that a retinal image of a white line represents a very good reflector for spreading induction. For this reason a retinal image or an after-image of a white line has been used as a screen. When an inducer is enclosed by a white line figure, only a small fraction of the spreading induction produced by the inducer can reach the outside of the white line figure. Such a protecting action of a white line figure was discovered by Motokawa[2] in 1951, and has been utilized extensively in various experiments of retinal induction, but the mechanism of the protecting action has remained obscure.

Summary

A process called "spreading induction" is started at the margin of a retinal image and propagated into the surrounding retinal areas. Thus a field of retinal induction is established around a retinal image.

1. The velocity of propagation was determined by measuring the latencies of the propagating process at various distances from the margin of a retinal image, and it was shown that the velocity depends greatly on the adaptation state of the retina, but only little on the intensity and hue of the light stimulus.

2. The value of velocity was 1.7 mm. per sec. in the dark-adapted retina and 42 mm. per sec. in the retina adapted to an illumination stronger than 1 lux.

3. When spreading induction comes across a white retinal image, it can totally be reflected at the margin of the retinal image. The critical angle of incidence for total reflection was determined, and on the basis of the law of refraction the velocity of spreading induction in the light-adapted part, the retinal image, was computed from the critical angle determined experimentally and the known velocity in the dark-adapted retina.

The so computed values were found to agree satisfactorily with those determined by the direct method.

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Liver Catalase Activity of Patients with Gastric Cancer

By

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Low liver catalase values in animals bearing malignant tumors have been reported by many workers[1], including Nakahara[2], who, making thorough investigations, isolated from the tumor tissue a substance causing a depression of liver catalase activity and designated it toxohormone. However, literature which deals with the liver catalase activity of human beings with malignant tumors is scanty.[3),4)] Thus, we made a comparative study of hepatic catalase values between patients with malignant tumors, especially with gastric carcinoma, and those with gastric or duodenal ulcer, with the results to be reported below.

Experiments and Comments

Methods

A wedge-shaped piece of liver tissue, weighing about 1 g., was removed from the margin of the left lobe immediately after laparotomy, and washed in physiological saline. A sample of 100 mg. in weight was taken from this piece, ground in a mortar, mixed with 10 cc. of phosphate buffer (pH 7.0), and centrifuged for 5 minutes at 3000 rpm. An aliquot of 0.3 cc. of the supernatant was taken in a vessel, to which was added 0.7 cc. of the buffer. Then, 10 cc. of 3 per cent hydrogen peroxide solution was added, and the mixture was shaken in a water bath at 37°C. The amount of oxygen generated for 5 minutes, which was to be used here as a measure of the catalase activity of the liver, was determined by means of a Battelli-Stern apparatus.

Results

In Table I are shown liver calalase values in 60 cases of malignant tumors (including 56 cases of gastric carcinoma, 1 of esophageal carcinoma,

1 of cardiac carcinoma, and 2 of carcinoma of the ampulla of Vater) and those in 24 cases of gastric or duodenal ulcer. It will be seen that in cases

TABLE I

Range of catalase values→	Number of cases belonging to each range of catalase values								Total cases	Average
	~4	4.1~5.0	5.1~6.0	6.1~7.0	7.1~8.0	8.1~9.0	9.1~10.0	10.1~		
Gastric carcinoma	1	18	24	9	4				56	5.42
Esophageal carcinoma & cardiac carcinoma			2						2	5.15
Carcinoma of the ampulla of Vater			2						2	5.08
Gastric or duodenal ulcer			1	5	8	5	4	1	24	8.11

TABLE II

Mode of operation ↓ Range of catalase values→	Number of cases belonging to each range of catalase values								Total cases	Average
	~4	4.1~5.0	5.1~6.0	6.1~7.0	7.1~8.0	8.1~9.0	9.1~10.0	10.1~		
Exploratory laparotomy	1	4	3						8	4.83
Palliative gastroenterostomy		5	1	2	1				9	5.40
Total gastrectomy			1	2	1				4	5.51
Gastrectomy		8	19	5	3				35	5.54

TABLE III

Size of tumors ↓ Range of catalase values→	Number of cases belonging to each range of calalase values								Total cases	Average
	~4	4.1~5.0	5.1~6.0	6.1~7.0	7.1~8.0	8.1~9.0	9.1~10.0	10.1~		
~10 cm.			1	1					2	6.13
10~7 cm.		2	9	3					14	5.49
7~4 cm.		2	6	1	1				10	5.59
4 cm.~		4	3		2				9	5.50

of various forms of carcinoma the liver catalase value was distinctly lower than that in cases of ulcer.

In addition, the relation of the catalase activity with the mode of operation selected according to the extent of gastric cancer and with the size of malignant tumors in excised specimens are shown in Tables II and

III, respectively.

Summary

The liver catalase activity of patients with malignant tumors was distinctly lower than that of patients with gastric or duodenal ulcer.

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The Site of Electrical Excitation of the Human Eyes by the Sinusoidal Alternating Currents at Frequency of 20 CPS

By

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If the threshold voltages of alternating currents passing through the head, as determined with flickering phosphenes as an index of excitation, are plotted as a function of frequencies, there is found a distinct minimum at a frequency around 20 cps on the electric threshold strength-frequency-curve provided that measurements are made with the partially light-adapted eye.

Barnett[1] stated that this frequency around 20 cps should be the optimum frequency for electrical stimulation of those portions of the optic tracts connected to cones. But agajnst his inference, much work has been done to ascribe the changes in electrical excitability of the eye for 20 cps to rod mechanism. Mita and Abe[2] and Abe[3], studied the effects of dark adaptation upon the electric threshold for square waves of 20 cps. They maintained that 20 cps may be the optimum frequency for electrical stimulation of those neural elements connected to rods. This view was supported by Meyer-Schwickerath[4] from location of flickering phosphenes at the periphery of the retina. Motokawa and Ebe[5] investigated by means of Motokawa's ζ-value the resonance curve and the spectral response curve, and they confirmed that the frequency of alternating current at 20 cps. must surely be concerned with the rod mechanism.

Yonemura and Nango[6] found an important fact that a single rectangular pulse of about 25 msec. in duration has an effect of stimulation similar to those effects obtained by repetitive stimulations at 20 cps, and later Nango[7], using this single pulse of 25 msec. as a stimulus, made an experiment for comparison between the electrical excitability-wavelength curve and the scotopic visibility curve. His experimental result showed that maxima of both curves were situated equally at about 510 mμ. Motokawa, Ebe, Arakawa and Oikawa[8] and Oikawa and Kurosawa[9] reported that the spectral response curve of rods has a maximum at 507 mμ, and that the

maximum of the curve does not show any shift even when the intensity of light is increased to such a high level of more than 100 times as high as the light threshold for the fovea.

In view of these experiments, it is likely that rods play still a dominant role for determination of the electric threshold for 20 cps. or a rectangular pulse of 25 msec. even at intensities of the adapting light extending to such a high level that vision becomes fairly photopic. Then, a question arises as to why cones have little participation in determining the electric threshold of the eye for 20 cps.

Recently, however, Mita, Fujimaki and Yaegashi[10] studied the dependence of the electric threshold upon intensities of the white adapting light. From this experiment they assumed that the structures stimulated by 20 cps. within the retina may be the bipolar cells. According to Polyak's synaptic anatomy[11], some kinds of bipolar cells connected principally to rods such as mop bipolars receive discharge impulses also from cone cells, when cones are activated by the light stimulus. Then it must be expected that both rods and cones would influence the electric threshold. Therefore, if some influences due to participation of cones can be revealed, it is of first importance to detect them in the experimental conditions, where different monochromatic rays are used as the adapting lights and their intensities are raised sufficiently high above the cone threshold. Such experiments, however, have not yet been performed. In order to secure the precise data regarding this problem, the present experiments were undertaken, with special attention to the color threshold of the adapting light.

Experimental

Methods

A tungsten filament of a 100 Volt–500 Watt lamp was used as a light source. The voltage and current of the lamp were controlled manually by an experimenter. The variations in the current during the experiment were usually less than 0.4%. Narrow bands of spectrum used for selective adaptation of the eye were isolated with 16 metal-interference filters of Lange & Co. Sometimes, several glass filters were also in auxiliary use together with the formers. Radiant transmittance of these filters were calibrated by a spectrophotometer of Beckmann type. Data obtained were plotted in Fig. 1.

A pair of opal glass plates was located closely in front of the observer's eyes and these plates served as illuminating screens for adaptation of the eyes; when they were illuminated from behind by a light beam, the observer's retinae were illuminated almost uniformly over the whole field of vision. Radiance of the illuminating screen was measured by means of a vacuum thermopile of 5.1 volt/watt in sensitivity and a galvanometer of 5.4×10^{-8} volt/mm in sensitivity. In the case of the adapting light of 549 mμ in wavelength, the maximum radiance

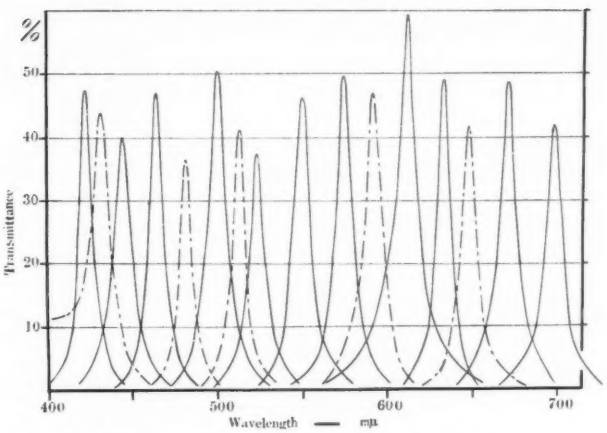

Fig. 1. Spectral transmittance of metal interference filters.

available for the experiment was 2.4×10^2 in relative unit, producing a luminance equivalent to about 4.0 Mlb.

The sinusoidal alternating current used for stimulation was generated by an oscillator of RC type. Three successive cycles of the alternating currents at 20 cps were given to the observer as the stimuli through an electronic rheotome devised by Mita, Fujimaki and Sato[12]. This rheotome enabled us to start the sinusoidal alternating currents at 0° phase and cease them at 360°. A silver plate of 3×2 cm² was inserted in the observer's mouth as a different electrode and another large silver plate was placed under the right sole as an indifferent electrode, being immersed in 1% saline.

Measurements of the electric threshold were made by the same procedure as reported in the previous paper[13]. The experiment was commenced after the observer stayed in darkness at least for 30 minutes. In the cases of light-adapted eyes, the measurement started after 10 minutes of light adaptation to every new level of the adapting lights, which, then, was raised successively. The value of electric threshold was determined by the method of trial and error.

Results

1) The electric threshold strength-log-radiance curve

The electric thresholds were measured at various radiances of the adapting light of different wavelengths. Data obtained were plotted as ordinates against log-radiance as abscissae in Fig. 2. Fig. 2 shows that all these curves, excepting the curve for wavelengths longer than 647 mμ, resemble greatly one another and each of them shifts horizontally by a certain distance depending upon wavelength of the adapting light. One curve for 499 mμ was taken as an example for description of its characteristics. As shown in Fig. 2, at low radiances between 10^{-5} and 10^{-3} unit the electric threshold for 499 mμ keeps constant with almost

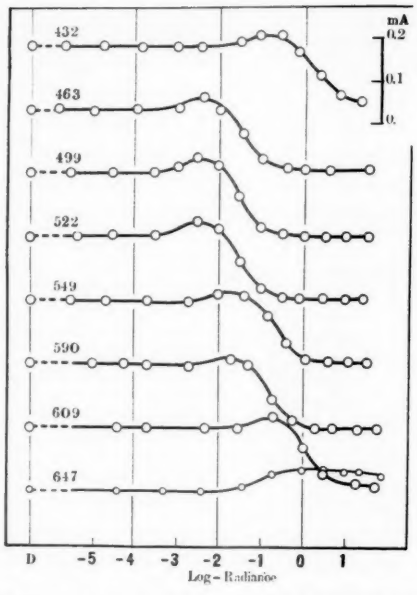

Fig. 2. Relation between electric threshold strength and radiance of monochromatic rays used as the adapting light. Ordinates: electric threshold in mA (each curve is plotted successively with a shift of 1.5 mA in ordinate). Abscissae: log-radiance in relative unit. D denotes complete dark. Numbers on the curves show dominant wavelengths of the adapting light.

the same value as that of the fully dark-adapted eye, then it increases slightly with increase in radiance, till it reaches a maximum at radiance of 3.11×10^{-2} unit. For convenience sake, this range of radiance from the light threshold to the level corresponding to the maximum was termed "section s". This "section s" is the range of colorless or purely scotopic vision, namely, within the "section s" no hue was elicited by the adapting light.

As the radiance was raised further, the electric threshold decreases rapidly to reach an inflection point (minimum) at radiance of about 10^{-1} unit, beyond which there is no further decrease. We termed this range of radiance corresponding to the descending part of the curve "section m". Within the "section m", color hue of the adapting light is noticeable, namely, this range belongs to mesopia, where both rods and cones are active at the same time. Then, the minimum value of the electric threshold is kept unchanged at radiances above 10^{-1} unit. This range of radiance was termed "section p", where vision is photopic. Thus, the electric threshold strength-log-radiance curve can be divided into three sections s, m, and p, according to the course of the curve. (see Fig. 4 C) It is noteworthy that the subjective sensation of hue and saturation elicited by the adapting light varies distinctly in conformity with changes in

electric threshold corresponding to the three sections s, m and p. All these rules mentioned above held for all other wavelengths with the exception of red rays.

Attention must be paid to the following two facts. The one is that within the range of " section p " the electric threshold reaches one and the same minimum value irrespective of wavelength of the adapting light, which amounts to about one-fourth of the dark value. The other is that the range of the " section m " for each monochromatic light is about 1.2 log unit (12.2 decibels), that is, one-third as narrow as compared with that range for white light, which amounts to about 3.6 log unit (36.2 decibels), as reported in the previous works of Mita *et al.* (10, 13). Consequently, the slope of the descending part of the curve for the white adapting light is far steeper than that for the monochromatic one, as shown in Fig. 4 C.

The curve for wavelength longer than 647 mμ differs slightly in shape from that for shorter wavelengths, that is, the hump on this curve is relatively large and broad. Appearance of the hue elicited by this long wavelength is out of accord with the rule mentioned above, namely, red color can be perceived even in the " section s "

2) The electric threshold strength-wavelength curve

The relations between the electric threshold and the wavelength of the adapting light were studied at 5 radiance levels of 3.11×10^{-3}, 2.0×10^{-2}, 4.13×10^{-1}, 2.68×10^{0} and 8.1×10^{1} of the adapting light. Curves obtained were illustrated in Fig. 3.

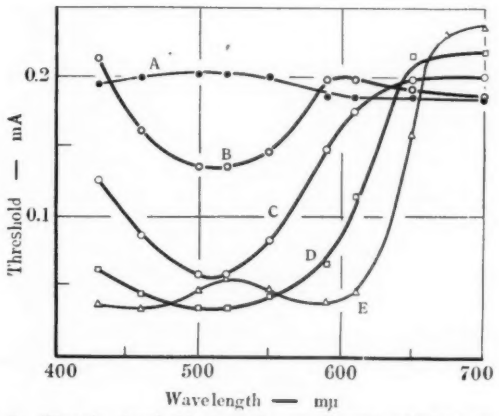

Fig. 3. Electric threshold strength—wavelength relations at 5 energy levels of the adapting light. Curves A, B, C, D and E show those relations obtained at radiances of 3.11×10^{-3}, 2.0×10^{-2}, 4.13×10^{-1}, 2.68×10^{0} and 8.1×10^{1} in relative unit respectively.

As shown in Fig. 3, the curve A for radiance at 3.11×10^{-3} unit has a slight curvature upward convex, a maximum (situating) at about 510 mμ. The curves B and C for radiances at 2.0×10^{-2} and 4.13×10^{-1} unit are U-shaped

within a range from 430 to about 600 mμ and the minimum for each curve of B and C lies samely at about 510 mμ. The curve D for $2.68 \times 10^{\circ}$ unit has a shape similar to the curve C, but the bottom of the U-shaped curve is far wider than that of C. The part of the curve D corresponding to the extreme red end of the spectrum rises higher than those parts of the other curves A, B and C. As the radiance of the adapting light is raised to 8.1×10^{1} unit, the electric threshold for wavelengths at the vicinity of 520 mμ begins to increase gradually, so that the curve E seems to be divided into two branches. In view of these facts, it is concluded that any shift of the minimum on the curve such as Purkinje shift could not be found.

In order to compare precisely the electrical excitability of the eye adapted to monochromatic rays with the photic excitability for one and the same rays, the electrical excitability (E) was calculated by the following formula; $E=(I_{\circ}-I)/I$, where $I_{\circ}$ and I are the electric threshold measured in mA of the fully dark-adapted eye and that of the eye sufficiently exposed to the monochromatic rays respectively. This measure E has the same meaning as "K" in Yonemura and Nango's experiment, where they did not measure the electric threshold in mA but in volt. Table I shows the values of E calculated from data of curve C in Fig. 3. As shown in Table I, the value of E reaches a maximum between 499

TABLE I

Wavelength in mμ	432	463	499	522	549	590	609	647	695
Electrical excitability in %	21.2	50.9	97.0	97.0	54.4	11.1	2.8	-2.9	-4.3
Scotopic luminosity in %	15.7	45.6	95.5	95.4	53.4	9.4	3.3	0.3	0.1

and 522 mμ and each value is fairly well consistent with the scotopic luminosity factor from Weaver[14], although some systematic deviations between them are found especially for red end of the spectrum. For wavelengths such as 647 and 695 mμ, E takes negative value. This fact means that the electric threshold of the eye rises to a certain value higher than the dark level (I°). It is interesting to note that the electrical excitability of the eye exposed to the red rays is depressed when cones dominate. This phenomenon can be found also in Yonemura and Nango's work.

DISCUSSION

Fig. 4 A shows a three-dimensional surface indicating the electric threshold as a function of log-radiance and wavelength of the adapting light. This diagram is based upon the data shown already in Fig. 2 and 3. On this surface, the electric threshold - log-radiance curves for given wavelengths are represented by heavy curves extending from left-rear to right-front. Curve H presents the locus connecting the maxima on the heavy curves, and curve L is the locus connecting the inflection points

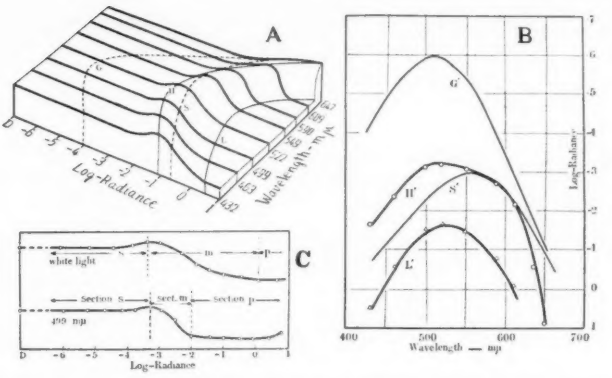

Fig. 4 (A). Three-dimensional surface showing the electric threshold as a function of log-radiance and wavelength of adapting light. Curve H is locus of the crest on strength—log-radiance curves (heavy curves). Curves L is locus of inflection points from descending part to horizontal part on the heavy curves. Curve G and S indicates colorless and color threshold of adapting light respectively. (B) Curve G′, S′, H′ and L′ are projections of the curves G, S, H and L on to the log-radiance wavelength plane. Distance between both curves of G′ and S′ indicates photochromatic interval. (C) Diagrammatic illustration of three sections: scotopia (s), mesopia (m) and photopia (p). Compare the shape of the strength-log-radiance curve for white adapting light with that for monochromatic one.

from the descending part to the horizontal part of the heavy curves.

As both general (colorless) and specific (color) thresholds were measured with respect to the adapting lights of different monochromatic rays, two kinds of these light thresholds were also illustrated by two dashed curves G and S on the three dimensional diagram in Fig. 4 A respectively. When 4 curves, G, S, H and L, were projected on to the log-radiance—wavelength plane, they were indicated by G′, S′, H′ and L′ in Fig. 5 B respectively. Then, the distance between both curves G′ and S′ means the photochromatic interval, where rods alone are considered to be in action. As shown in Fig. 4 B, two curves S′ and H′ lie so near that they intersect each other at about 600 mμ. Therefore, it is regarded as probable that the maximum (crest) of the electric threshold strength-log-radiance curve corresponds to the color threshold approximately.

Generally speaking, excepting the case of red rays, we perceive the sensation of color hue elicited by the adapting light at first, soon after the electric threshold begins to decrease from the maximum (crest). Further increase in radiance brings about progressive increase in saturation of color, tending toward its highest degree at L′ level of the radiance, where

the electric threshold just reaches the minimum. In other words, within the range of radiance between H′-and L′-level the remarkable increase in saturation of colored light occurs simultaneously with the rapid decrease in the electric threshold for 20 cps.

Accordingly, it is natural to think that the decrease in electric threshold for 20 cps. would be concerned mainly with the cone-mechanism, as did Barnett in 1941. But, as mentioned above, Barnett's view was not shared by many investigators. The most important objection is based on the fact that the electrical excitability—wavelength curve for equal energy spectrum coincides with the scotopic luminosity curve, and the former curve does not show any Purkinje shift even in the case of sufficiently high intensity of the light stimulus. These facts may demonstrate domination of rod mechanism, but do not mean necessarily that rods themselves are stimulated by the current. Because, there is a decisive evidence that variations in electric threshold for 20 cps. due to varying adaptation of the eye do not run parallel with those variations in photic threshold due to the same cause. Therefore, it is likely that the site of excitation by the currents of 20 cps has little concern directly with the photoreceptor cells, but a great concern with the bipolar cells connected to rods, as has been assumed by Mita, Fujimaki and Yaegashi. Hence, if we assume that the electric threshold for 20 cps. depends mainly upon the rod process, it should be expected that the electric threshold for 20 cps. would vary much more remarkably at low radiances of the adapting light, where rods are active exclusively, than at such high levels as cones come into play. But, against the above mentioned expectation, this experiment shows that the electric threshold for 20 cps. remains approximately unchanged with the same value as in darkness within a fairly wide range of low radiance between 10^{-6} and 10^{-3} unit, where vision is entirely a function of rods. What is the explanation of this phenomenon? The question may be answered by further assuming that the decrease in electric threshold for 20 cps. takes place only when the radiance of the adapting light arrives at such a level that not only rods but also cones are active concurrently.

Then, it may be explained that the decrease in electric threshold does not occur at least in the purely scotopic vision where cones do not function. An opposite relation is found in the case of the extreme red rays of 647 mμ, where cones dominate. In this case, the decrease in electric threshold does not occur within a fairly wide range of radiance below about 10 unit, even though there is elicited distinct sensation of red hue. This fact indicates again that the photic excitation of sufficient rods is a necessary condition for reducing the electric threshold for 20 cps. The necessity of participation of rod mechanism in reducing the electric threshold was reported lately by Takahashi[15], who found that, in 4 cases of

pigmentary degeneration of the eye suffering from nyctalopia, any intensity of white illumination of the retina had no decreasing effect on the electric threshold for 20 cps., and that these events might be ascribed to affection of rod cells.

After all, it is concluded that the structures stimulated by the currents at 20 cps. may be those neurons which have connections to both rods and cones, and that at least the decrease in the electric threshold of those neurons may be produced by a peculiar summation effect brought about by both rod- and cone-mechanisms. This conclusion supports Mita's working hypothesis that the structures stimulated by the currents at 20 cps. may be bipolar cells.

Summary

Taking, as an index of excitation of the eyes, flickering sensation (electrical phosphenes) elicited by the sinusoidal alternating currents at 20 cps. passing through the head, the author studied precisely the relation of electric threshold of the eye to radiance of the adapting light of different wavelengths.

1. The electric threshold strength—log-radiance curve has a reversed S-shape. The course of the curve can be divided into three sections, each corresponding to three physiologically significant states of vision, that is, scotopia, mesopia and photopia.

2. The electrical excitability—wavelength curve for equal energy spectrum resembles greatly the scotopic luminosity curve in form.

As to the position of the crest on this curve, no shift takes place toward longer wavelength such as Purkinje shift even when the radiance of the adapting light is raised sufficiently.

3. Decrease in electric threshold of the eye for 20 cps. due to illumination with monochromatic rays is always accompanied with increase in saturation of the color, except for red. Both kinds of variations take place only within a relatively narrow mesopic range of illumination, where both rods and cones are in action concurrently.

4. These results support Mita *et al's* view that the structures stimulated by the currents at 20 cps. may be bipolar cells.

I wish to express my cordial gratitude to Prof. T. Mita who has given me valuable advice throughout the experiments and constructive criticism during the writing of this paper.

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A Study of a New Antitumor Substance, Cellostatin

I. On the Isolation and Some Properties of Cellostatin

By

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In recent several years, attempts to search antitumor substance in actinomycetes antibiotics have been tried, and it has been confirmed that the culture filtrates of some streptomyces strains produce the active agents on the growth of a variety of mouse and rat tumors. The study on the screening of these substances necessitates the rapid, sensitive, efficient and quantitative methods by which the antitumor activity is estimated.[1] Also, the methods are required strongly from stand points of extraction of the active agents in the metabolic filtrates of streptomyces.

Although the correlation between antibacterial and antitumor effects are not able to be prematurely concluded[2], some substances[3], for example Azaserine[4] and Puromycin[5] may possess both, antimicrobial and antitumor properties. Consequently, it is worthy to utilize its antimicrobial activity as an indicator for the isolation of the tumor-inhibiting agents.

The author has isolated a new antitumor substance " Cellostatin " produced by a strain of Streptomyces against the Ehrlich ascites carcinoma, which is active also to some yeast and fungi. Therefore, the isolation of antitumor agents was plotted with the antifungal activity as an indicator.

In this paper, isolation and some properties of this substance are reported.

Experimental

Taxonomic Studies

A strain (our collection No. E-150) of Streptomyces was isolated from soil samples collected at Omiyamachi, Ibaragi prefecture. The strain was a species of streptomyces and seemed to be related to *Str. flocculus* at first. However, its soluble pigment in organic media was brown to dark brown, and in this respect it has no similarities to any of the streptomyces

species hitherto described. Some similarity was able to be found in the description of *Str. lavendulae*. But in the respects, such as it does not produce a lavender color and characteristic spirals, this strain differed from *Str. lavendulae* too. The results of the mycological studies are shown in Table I. From the reason mentioned above cellostatin-producing strain, E-150, is deemed to be a new species and is named *Str. cellostaticus*, n. sp.

Production of the Substance

The antibiotic was produced in the medium containing 2.5% starch,

TABLE I

Mycological Properties of No. E-150 Strain

	Media	Growth	Aerial mycelium	Soluble pigment	Remarks
Cultural characteristic	Glucose-asparagine agar	Colorless to pale yellow (7-19-2)	Thick, abundant, cottony like, white to reddish grey (1-16-1)	None	
	Nutrient agar	Dark brown (4-12-2)	White to greyish white	Blackish brown	
	Yeast extract agar	Some elevated, pale yellowish brown	Abundant, thick, cottony, light purplish gray (24-17-1)	Faint brown	
	Ca-malate agar	Colorless to pale yellow (7-19-2)	abundant, velvety, light purplish grey (24-18-1)	None	
	Czapek's agar	Glistening, pale yellowish brown (6-18-3); center of colony elevated	White	None or dull yellow orange (6-18-4)	
	Starch agar	Thin, colorless	Abundant, white	None	
	Potato	Spreading, thick, much wrinkled, blackish	Abundant, cottony, white	Color of plug: Black	
	Carrot	Spreading, thick, wavy, faint brown	Abundant, white	Color of plug: Dark brownish	
	Egg media	Very much wrinkled in margin, dark yellowish brown (7-13-2)	White in center	Dark brown	

	Gelatin	Thin, colorless	Thin, white	Brown colored liquefied portion	Liquefaction: about 10.1 mm of depth of gelatin in tube is liquefied in 30 days
	Milk 37°C				Coagulation: Rapid (7 days), clot formation. Peptonization: Rapid (8-15 days), clearing up all the milk. Change of reacto: Alkaline (pH 8.0)
	Tyrosine medium				Tyrosinase: not formed
	Czapek's solution	Ring formation on the dry portion of tube, thick colorless	abundant, some powdery, white to grayish white	Pale orange (4-19-3)	Nitrites formation: Excellent
Morphology	Spirals	Glucose-asparagine agar: None, a few spirals may be formed at times			
	Conidia	Spherical or oval.			
Utilization test of various carbon sources	Utilization	Rhamnose, lactose, raffinose, inulin, inositol, sodium citrate, L-xylose, maltose, glycerol, glucose, starch, sucrose, D-fructose, D-mannitol, L-arabinose, sodium acetate			
	No utilization	Phenol, D-sorbitol, dulcitol, sodium succinate, D-galactose, salicin			

3.5% soy bean meal, 0.5% dried yeast, 0.25% NaCl, 0.005% $MnCl_2 \cdot 4H_2O$, 0.0005% $CuSO_4 \cdot 5H_2O$, 0.005% $ZnSO_4 \cdot 7H_2O$, 0.35% $CaCO_3$, (pH 7.2) under the shaking condition.

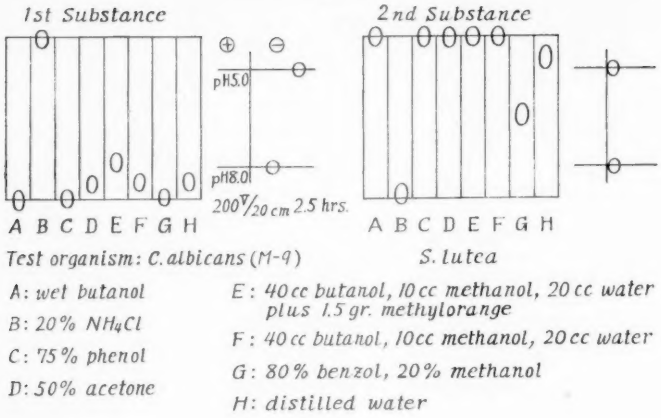
The potency was determined by the pulp disc method and broth dilution method with *Candida albicans* (M-9) as test organism. But the border line of the inhibition circle produced by cellostatin on candida plate was turbid and indistinct. So the diffusion method was not accurate for assay.

The maximal production of the antibiotic was obtained on the 4th to 5th day of incubation with pH 7.4; its potency was 1:8–16 dilution unit against *Candida albicans*, and about 200mcg/m*l* of final substance was obtained from this filtrate.

Purification

The mycelium was removed from the fermentation broth by filtration at pH 6.0. The clear filtrate was passed through IRC-50 cation exchange resin column (hydrogen form) corresponding to 1/30 volume of

Fig. 1


1st Substance
2nd Substance
pH5.0
pH8.0
200V/20cm 2.5 hrs.
A B C D E F G H
A B C D E F G H
Test organism: C. albicans (M-9)
S. lutea
A: wet butanol
B: 20% NH4Cl
C: 75% phenol
D: 50% acetone
E: 40cc butanol, 10cc methanol, 20cc water plus 1.5 gr. methylorange
F: 40cc butanol, 10cc methanol, 20cc water
G: 80% benzol, 20% methanol
H: distilled water

the filtrate at pH 7.5 by s.v. 5–6 in order to adsorb almost all active agents. The column was washed with water to remove impurities, and the substance was eluted with 80% aqueous acetone, and further with 80% acetone containing 0.2 *N* HCl at s.v. 1–2. At that time, 2nd substance (active against *S. lutea*) was eluted mainly in 80% aqueous acetone fraction, while 1st substance (active against *C. albicans*) in 80% acetone containing hydrochloric acid.

As shown in the summarized and electrophoretic papergram (Fig. 1), these two substances are different from one another. By the further experiments of antitumor activity, it was confirmed that the 1st substance exhibited the destructive effect against Ehrlich ascites carcinoma, while the 2nd substance no activity. Consequently, the extraction of antitumor agents was followed using the antifungal activity as an indicator; the 1st substance was designated Cellostatin. The potent fractions eluted by 80% acetone containing hydrochloric acid were combined, adjusted to pH 6.0 with NaOH, concentrated in vacuo, and dried. The brownish residue was extracted with anhydrous methanol, and the insoluble impurities were filtered off. The methanolic extract was dried in vacuo and again taken up in an hydrous methanol. These processes were repeated two or three times. The resulting active residue was dissolved in acidic methanol and precipitated with acetone. The crude antibiotic was further purified by Al_2O_2 and carbon (Darco G-60) chromatography after pretreatment with H_2SO_4 according to Cartor *et al.*[6] In this way, sulphate of Cellostatin was obtained as a colorless platelet crystals. Crystalline reineckate (d. p. 156–166°C) was also isolated from the pure H_2SO_4 salt.

Chemical and Physical Properties

Cellostatin was a basic substance, very soluble in water, slightly soluble in methanol, but insoluble in ethanol, butanol, acetone, ether, and other organic solvents. It is precipitated from its aqueous solution by picric acid, Reinecke acid, and phosphotungstic acid. The antibiotic is very stable at pH 7.0, 5.0 and 2.0 and relatively unstable at pH 9.0.

The sulfate crystal is colorless platelets and the melting point of its reineckate is 156–166°C (decomp.). It indicated positive reaction in ninhydrin, Sakaguchi and Molisch, but the negative in biuret, Millon, Fehling, Maltol, $FeCl_3$, and Elson-Morgan test. The elemental analysis indicated: C 22.10%, H 4.52%, N 13.15%, O 27.77%.

As shown in Fig. 2, the ultraviolet absorption spectrum represented that max $E^{1\%}_{1cm}$ value is 115 at 265 mμ and min $E^{1\%}_{1cm}$ value 98 at 245 mμ in distilled water. The infrared absorption spectrum of the antibiotic is shown in Fig. 3.

Fig. 2

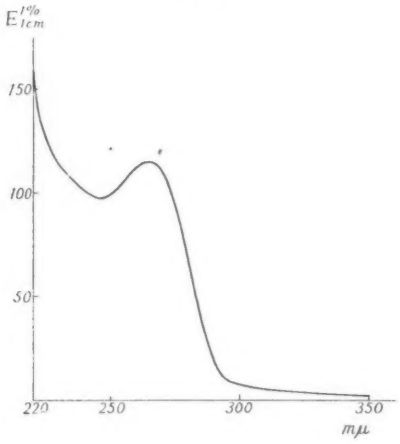
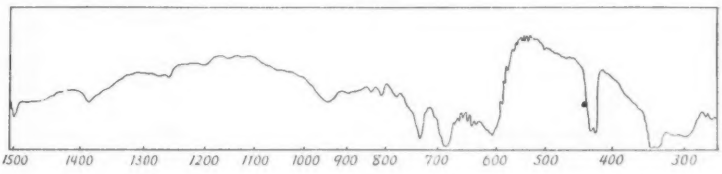


Fig. 3



Biological Properties

Antimicrobial spectrum of Cellostatin by agar streak dilution method is shown in Table II and III. The antibiotic was found slightly active against some yeast and fungi. It also indicated static action against *Trichomonas vaginalis* at the concentration of 100 mcg/m*l*.

The LD_{50} for mice by single intraperitoneal injection is about 15 mg/kg. The daily injection of 2 mg/kg for one week into mice did not exhibit any toxic signs. The histological findings of toxicity were examined in the autopsied mice after lethal dosis injection. It was characterized by the advanced parenchymatous degeneration and haemorrhage in liver and kidney.

Summary

Streptomyces Sp. No. 150 produced two antibiotics in the culture broth, which were separated from one another with a cation exchange resin. The first substance was isolated as sulfate in a crystalline form, and characterized as a basic, water-soluble, antifungal substance with anti-

Table II

Antibacterial Spectrum

Test organism	MIC μg/m*l*
M. pyog. v. aureus (*Terashima*)	100<
M. pyog. v. albus	100<
M. citreus	50
B. subtilis (NRRL-558)	100<
B. anthracis	100
S. lutea	100
E. coli (Umezawa)	100<
,, SMR	100<
S. typhosa	100
S. paratyphi A	100
S. paratyphi B	100
S. enteritidis	100<
Sh. dysenteriae	100
Sh. sonnei	100<
P. aeruginosa	100
P. vulgaris (OX-19)	100
*Cl. welchii**	100<
*Cl. tetani**	100<
*Cl. botulinum**	50

* Tested in Tarozzi's broth.

TABLE III
Antifungal Spectrum

Test organism*	MIC mcg/ml
Sacch. cerevisiae	100<
Zygosaccharomyces	100<
C. albicans (M-2)	25
,, (M-8)	100
,, (M-9)	25
,, (M-10)	100
,, (Takeda)	25
C.krusei	100<
C. parakrusei	100<
C. tropicalis	100<
C. pseudotropicalis	25
Torula	100
Mycotorula	100<
Rhodotorula	100<
Willia	100<
Asp. niger	100
Asp. oryzae	100
Trich. interdigtalis	12.5
,, *gypseum*	100
,, *purpuralis*	6.3
Mucor pusils	100

* Tested in 1% glucose nutrient agar.

tumor activity. The antibiotic seems to differ from any other formerly reported antifungal substances.[7] It was recognized as a new antibiotic and named " Cellostatin ". The second substance is active to *S. lutea*, soluble in organic solvent, and inactive for the growth of tumors.

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A Study of a New Antitumor Substance, Cellostatin

II. On Antitumor Effects of Cellostatin

By

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(Received for publication, October 2, 1957)

In previous paper[1], the isolation and some properties of cellostatin which was produced by a strain of streptomyces and is active to some kind of yeasts and fungi were described. In the present paper, antitumor activity of this substance will be reported.

Experimental

Materials and Methods

Ehrlich carcinoma strain was transferred to the highly inbred mice, dd-D, which weighed about 20 g.[2] The ascites containing the tumor cells were taken from the mice in which the same tumor cells had been inoculated 7 days before. It was diluted with sterile saline to contain ten million cells per milliliter, and 0.1 m*l*. of this cell suspension was inoculated intraperitoneally to mice. In each experiment, 5 or 10 mice were used as a group for treatment and control. From 24 hours after the inoculation, 0.5 m*l* of the sterile culture filtrate of streptomyces E 150 after 4 days shaking culture or the physiological salt solution of cellostatin was injected once a day for 5–10 days into the peritoneal cavity, while the same volumes of physiological salt solution were injected to the control groups. The daily injection of the drug was started 7 days after inoculation in the second experiment.

The antitumor effect[3] was determined from the following three standpoints.

1) The prolongation of the survival period of treated mice. The control mice died usually 10–15 days after the inoculation of 10^6 tumor cells, and the day when 80 per cent of the animals died of tumor growth was checked. On the other hand, the treated mice also were checked at the day of 80% death. Thus, the prolongation of the survival periods was estimated from

the survival index $\frac{\text{the day when 80\% of treated mice has died}}{\text{the day when 80\% of cotrol mice has died}}$.

When the survival index was larger than 1.5, the filtrates were deemed to be effective against cancer.

2) *The inhibition of the ascites increase and solid tumor development.* The dead animals were examined by autopsy in the respects of the ascites increase and solid tumor growth in the inoculated sites of subcutaneous tissue and in the peritoneal cavity. The solid tumors and other cancerneous lesions in omentum, mesenterium, retroperitoneum, intrapelvic fat and others were macroscopically observed, and those of treated and control mice were compared with one another.

3) *The cytological observation.* 5 or 7 days after the transplantation, the smears of the ascites were made and the cytological changes in tumor cells were microscopically examined. Then the test of direct effects of the the drug on tumor cells was conducted, i.e., after the single injection of drug the rate of cells in mitosis in ascites of 7 day-old-cancer was counted, and its decrease was checked at regular intervals after treatments.

Results

Antitumor Activities of Culture Broth

The No. 150 strain was shake-cultured in three kinds of culture media : the one contrained 2.0%, glycerol, 0.5% meat extract, 0.5% peptone and

Table I

Antitumor Activity of the Culture Filtrate of No. 150 Strain

Mice No.	Injection			Survival		Mitotic rate	Amount of ascites m/.	Solid tumors	
	Dosis m//day	Times	Total m/.	Days	Index			Subcutan	Peritoneal cavity
1	0.5	5	2.5	48 scf*			0	−	−
2	0.5	2	1.0	3			0	−	−
3	0.5	4	2.0	48 scf†	3.2<	0	0	±	−
4	0.5	4	2.0	20			0	−	±
5	0.5	5	2.5	19			tr.	+	±
1				13			2.0	++	±
2				16			4.8	+	±
3	Control			15†	1.0	1.6	3.5	+	±
4				15			0.5	+	±
5				15			1.5	−	±

*: The day when the animal was sacrificed. †: The day when 80% of the animals has died of tumor growth.

0.5% NaCl (pH 7.0), and the others were substituted with glucose or starch for glycerol, respectively. After 4 days' shaking culture at 27°C, the culture filtrates of three kinds of media were combined, and its anti-

TABLE II

Antitumor Activity of Cellostatin Against 1-day-old Tumor

Group	Treatment			Mice No.	Survival		Amount of ascites		Solid tumor	
	Dosis mcg/day	Times	Total mcg		Days	Index	ml.	Average	Subcutan.	Peritoneal cavity
1	50	3	150	1	(8)	1.3	0	0.7	−	−
				2	21		1.0		++	+
				3	27		3.0		++	+
				4	13		0		±	−
				5	14		tr.		−	−
				6	(7)		0		−	−
				7	(7)		0		−	−
				8	19*		1.0		±	−
				9	16		2.0		+	+
				10	(7)		0		−	−
2	25	5	125	1	13	2.1	2.0	1.6	−	++
				2	32*		1.0		++	+
				3	22		1.0		++	+
				4	13		1.0		+	−
				5	20		5.0		++	++
				6	33 scf.		3.0		++	±
				7	20		1.0		++	+
				8	33 scf.		0.7		−	+
				9	31		0.8		++	−
				10	17		0.5		+	+
3	10	10	100	1	28*	1.9	1.5	1.4	+	+
				2	16		2.0		−	++
				3	18		3.0		++	++
				4	29		tr.		++	−
				5	11		0.7		−	±
				6	33 scf.		0		++	−
				7	12		1.0		−	+
				8	20		2.5		++	+
				9	17		2.5		+	++
				10	14		1.0		−	++
Control				1	13	1.0	1.5	2.3	−	+
				2	11		1.0		−	++
				3	15*		3.0		+	++
				4	12		1.5		+	++
				5	15		1.0		−	++
				6	15		3.5		++	++
				7	11		1.0		+	+
				8	9		3.0		−	+
				9	12		3.0		±	+
				10	13		4.0		+	+

( ): The mice that had died earlier than those of the control groups due to the toxicity of the antibiotic. *: The day when 80% of the animals had died. scf: The day when the animal was sacrificed.

tumor activity was tested. As shown in Table I, the culture broth of No. 150 strain suppressed the increase of ascites and prolonged the survival periods of inoculated mice (tumor cell : 2.3×10^6) by daily intraperitoneal injection after 24 hours from the inoculation. But Nol 2 mouse died at 3 days after 2 times treatments. Therefore, it will be presumably assumed, that the culture filtrate of No. 150 strain may exhibit some toxic signs in this dosis. On the other hand No. 1 and 3 mice indicated marked prolongation of survival periods, and were sacrificed at 48 days after inoculation. It was confirmed that the 150 strain constantly produced the antitumor substance in the further repeated tests.

Antitumor Activity of Purified Cellostatin

1) The effect of daily injection against 1-day-old tumor. After 24 hours from the inoculation (1.8×10^6 cell), cellostatin was injected in amounts of 50 mcg/0.5 ml, 25 mcg/0.5 m*l*, and 10 mcg/0.5 m*l* intraperitoneally one time a day for 3, 5 and 10 days respectively, using 10 mice regarding each dosis. The results are shown in Table II. In each dosis, Cellostatin suppressed the increase of ascites and prolonged the survival periods. But group 1 mice exhibited only a little prolongation due to the toxicity of the drug, and 4 mice in group 1 (10 mice) died with no signs of tumors earlier than those of control group. Other 6 mice of this group were somewhat prolonged in their survival days with little or no signs of tumor lesions, especially of ascites tumors in comparison with control animals. On the other hand the mice of group 2 and 3 were markedly prolonged in their survival days, but indicated no or little inhibition of the tumor lesions, especially no effect upon the growth of the solid tumors.

2) The effect of daily injection against 7-day-old tumor. In the experiment shown in Table III, the daily injection of Cellostatin was started 7 days after the inoculation of the tumor cells (1.6×10^6). The ascites increase was inhibited in the treated group, but the survival period was scarcely prolonged in 2 mice of 10 in group 1 which exhibited a marked retardation of death. The degree of tumor lesions indicated generally no or little difference in these 3 groups.

Tumor Cells Treated with Cellostatin

The influence of cellostatin upon the rates and forms of mitosis was tested with the 7-days-old tumor cells (Ehrlich ascites carcinoma). The rates of mitosis were decided by the ratio of the number of mitotic cells per 100 cancer cells before treatment at regular intervals to those after a single intraperitoneal injection of 200 and 100 mcg. of cellostatin respectively. As shown in Fig. 1, the mitosis of Ehrlich ascites tumor cells decreased most distinctly 3 to 6 hours after the drug administration. Thereafter it re-

TABLE III

Antitumor Activity of Cellostatin Against 7-day-old Tumor

Group	Treatment: Dosis mcg/day	Treatment: Times	Treatment: Total mcg	Mice No.	Survival: Days	Survival: Index	Amount of ascites: m/.	Amount of ascites: Average	Solid tumor: Subcutan.	Solid tumor: Peritoneal cavity
1	50	2	100	1	9	1.2	1.4	0.9	−	+
				2	12		1.5		−	++
				3	11		1.0		±	++
				4	43		0		++	+
				5	53		1.5		++	±
				6	15*		1.5		−	++
				7	9		2.1		±	+
				8	11		1.0		−	++
				9	10		0.4		±	+
				10	10		0.5		±	++
2	25	3	75	1	15*	1.2	0.8	0.8	+	+
				2	10		0		−	−
				3	11		0.7		±	++
				4	14		1.7		+	++
				5	17		1.0		+	++
Control				1	13*	1.0	5.5	3.4	+	++
				2	10		0.8		−	++
				3	12		1.5		−	++
				4	10		2.5		+	++
				5	16		6.5		+	++

*: The day when 80% of the animals had died.

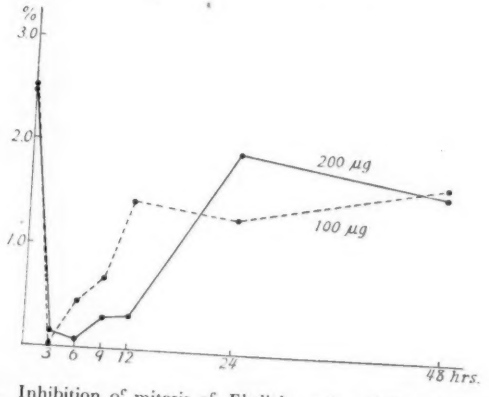

Fig. 1. Inhibition of mitosis of Ehrlich ascites 7-day-old tumor cells by Cellostatin.

covered gradually, and reached almost the normal range of mitotic rate after 24 hours. Therefore, the duration of its influence was limited to a relatively short time.

SUMMARY

An antitumor agent was produced by a strain of streptomyces, and its extraction was plotted with the antifungal activity as an indicator, Its destructive effect against ascites type of Ehrlich mouse carcinoma cells is correlated with its antifungal activity. But it needs scarcely to be said that the antifungal substance does not always inhibit the growth of tumors.[4] In the previous paper[5] the authors screened 273 strains of streptomyces for their production of antitumor substances prolonging the life of mice inoculated with Ehrlich carcinoma. Out of these 11 strains which produced constantly such a antitumor agent, contained 5 strains which exhibited no antimicrobial activity. Nevertheless, it may be still worthy and convenient to utilize its antimicrobial properties if the both biological activities are correlated with one another. In the intraperitoneal administration of the antibiotic, it was proved that the ealier the treatment was given, the more effective the drug was. So Cellostatin suppressed the increase of ascites, and prolonged the survival periods with little or no inhibition of the solid tumor growth in the early treatment. In the cytological observation, it was seen that the destruction of the ascites tumor cells and the prolongation of survival periods of the treated mice were remarkable. These facts should be due to its direct destructive activity upon the cancer cells.

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On the Interrelation of the Specific Gravity of the Spinal Fluid with Its Contents of Electrolytes and Total Protein

By

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(Received for publication, October 19, 1957)

There are only very few reports treating the fluctuation of the content of electrolytes in the spinal fluid in the cases of neural diseases. The same may be said, in these days, on the theme of the specific gravity of the liquor.

Recently, the N-N Type Dilution Hydrometer[1] was invented by Nakazawa and Nakai of this clinic, which has made it possible to measure the specific gravity of liquid samples available only in small quantity with precision, furnishing us with a promising weapon to attack the problems concerning the specific gravity of the spinal fluid.

By the aid of this device, we tested 153 samples of spinal fluid for making investigations on the specific gravity and its interrelation with the electrolyte and the total protein content of the liquor, and also measured the rise and fall of the specific gravity and the electrolyte and the total protein content of the spinal fluid in neural disease cases following the current practice of alterative treatments, such as fever therapy, electric shock therapy and blockade of Ganglion stellatum.

Experimental

Method

The specific gravity of the spinal fluid was measured with an N-N dilution hydrometer. One or two cc. of the liquor was diluted to the appropriate volume with distilled water and corrected to the temperature of 15°C for calculating the standard value of its specific gravity.

Of the electrolytes, the Na and K contents were measured in samples of 0.3 cc. of spinal fluid diluted to 50-fold in volume with redistilled water with the Beckman's spectrophotometer, Model DU with flame attachment[2], using standard samples prepared in step-wise adjusted concentrations as

controls, and the Cl content in 0.1 cc. samples diluted to around 20-fold with redistilled water, following the Schales and Schales's mercuri-metric determination of chloride[3].

For measuring the total protein content, 2 cc. of spinal fluid was sampled and subjected to turbidimetry, using a Beckman's spectrophotometer.

The samples of spinal fluid of 10 " normal subjects " (herein, normal subjects include patients of diseases not affecting the neural system) were taken on the occasions of effecting lumbar anaesthesia in surgical operation. The subject cases of diseases were divided into the 7 major groups of hypertensives, neurotics, syphilitic cases, cases with cerebrospinal tumors, cases with various meningitis, encephalitis japonica cases and case of miscellaneous other neural diseases, comprising both hospitalized and visiting patients.

Following alterative treatments, the samples of spinal fluid were taken from in-patients on the next day after the completion of a round of fever therapy, 2 hours after the electric shock and 30 min. after the blockade of Ganglion stellatum.

Results

Specific gravity, electrolyte and total protein content of spinal fluid. The maximum, minimum and average values of these items and their deviation from normal values observed in the 7 groups above were as shown in Table I.

The specific gravity and electrolyte contents before and after specific treatments :

The values observed in patients subjected to the therapeutic operations specified above were as shown in Table II, III and IV.

Discussion

There has been little disputes on the specific gravity of the spinal fluid in the past, only the range of 1.006–1.008 being indicated without further definition. Recently, Nakai and Ikeda[5] of this laboratory undertook a nearer examination of these values using an N-N dilution hydrometer with samples obtained from about 200 subjects, and have reported that the normal values stand between 1.0070 and 1.0080, values exceeding the latter always indicating pathological cases. In all my tested cases too, the normal subjects showed the values ranging between 1.0075 and 1.0080, the cases with neural diseases always showing values above 1.0081.

On the contents of electrolytes in the spinal fluid in the cases of diseases of the neural system there have been a number of reports[6)–14)], the oldest being that by Mestrezat[15], but these reports contained only data obtained by chemical analyses. Since the flame spectrophotometer came into use,

TABLE I

Disease	No. of cases		Specific gravity	Na mEq/L	K mEq/L	Cl mEq/L	Total protein mg/dl
Normal	10	Range	1.0075 —1.0080	122.0 —154.5	2.5 —3.3	111.2 —138.3	10 —62
		Average	1.0078	137.9	2.8	124.3	30.7
Essential hypertension	15	Range	1.0076 —1.0095	132.0 —158.0	2.2 —3.2	107.5 —141.3	18 —74
		Average	1.0084	145.8	2.8	126.3	37.5
		Deviation	+0.0006	+7.9	±0	+2.0	+ 6.8
Neurosis	26	Range	1.0078 —1.0090	130.0 —158.0	2.6 —3.4	102.5 —151.0	11 —64
		Average	1.0084	144.7	2.8	125.2	31.4
		Deviation	+0.0006	+6.8	±0	+0.9	+ 0.7
Syphilitic disease	28	Range	1.0079 —1.0094	131.0 —160.0	2.5 —3.5	114.9 —143.8	15 —324
		Average	1.0085	143.7	2.9	126.8	53.3
		Deviation	+0.0007	+5.8	+0.1	+2.5	+22.6
Cerebral & spinal tumor	9	Range	1.0079 —1.0091	134.5 —154.0	2.5 —3.7	111.3 —139.7	16 —106
		Average	1.0084	143.7	3.0	125.1	52.6
		Deviation	+0.0006	+5.8	+0.2	+0.8	+21.9
Meningitis	5	Range	1.0082 —1.0093	143.0 —151.0	2.5 —3.0	120.3 —136.2	54 —126
		Average	1.0085	148.3	2.8	128.3	73.0
		Deviation	+0.0007	+10.4	±0	+4.0	+42.3
Encephalitis japonica	8	Range	1.0076 —1.0087	136.0 —151.0	2.1 —4.9	110.9 —130.9	38 —127
		Average	1.0082	144.0	2.9	120.1	79.0
		Deviation	+0.0004	+6.2	+0.1	−4.2	+48.3
Diseases of nervous system	37	Range	1.0072 —1.0097	119.0 —160.0	2.1 —3.6	113.7 —140.4	14 —137
		Average	1.0084	143.9	2.9	126.6	40.0
		Deviation	+0.0006	+6.0	+0.1	+2.3	+ 9.3

TABLE II

Fever Therapy

Patient Name	Sex	Age	Diagnosis		Specific gravity	Na mEq/L	K mEq/L	Cl mEq/L	Total protein mg/dl
1) M.F.	♂	34	Cerebral syphilis	before	1.0082	133.5	2.8	127.8	55
				after	1.0090	137.0	2.9	134.2	17
2) C.E.	♂	35	Traumatic neurosis	before	1.0081	130.0	2.7	119.8	11
				after	1.0085	148.0	2.7	125.8	30
3) Y.K.	♂	19	Syphilis	before	1.0084	137.0	3.0	117.5	48
				after	1.0090	138.0	3.0	121.6	50
4) K.H.	♂	45	Tabes dorsalis	before	1.0084	138.0	3.3	121.6	30
				after	1.0086	141.5	3.8	120.0	28
5) S.H.	♀	35	Neurosis	before	1.0083	140.5	2.8	129.7	24
				after	1.0084	149.5	3.0	127.4	27

TABLE III
Electric Shock

Patient Name	Sex	Age	Diagnosis		Specific gravity	Na mEq/L	K Emq/L	Cl mEq/L	Total protein mg/dl
1) H.T.	♀	29	Neurosis	Before	1.0080	140.0	3.0	125.5	31
				After	1.0083	145.0	2.7	127.5	27
2) G.H.	♂	39	Neurosis	Before	1.0082	140.0	2.7	114.4	50
				After	1.0083	141.5	2.7	117.9	39
3) K.S.	♀	28	Neurosis	Before	1.0080	130.5	2.8	121.2	32
				After	1.0090	138.5	2.8	121.9	30
4) T.E.	♂	34	Neurosis	Before	1.0084	148.5	2.7	125.8	47
				After	1.0085	152.0	2.8	123.7	30
5) S.H.	♂	37	Neurosis	Before	1.0080	140.0	3.0	127.0	22
				After	1.0082	146.5	2.8	128.3	20

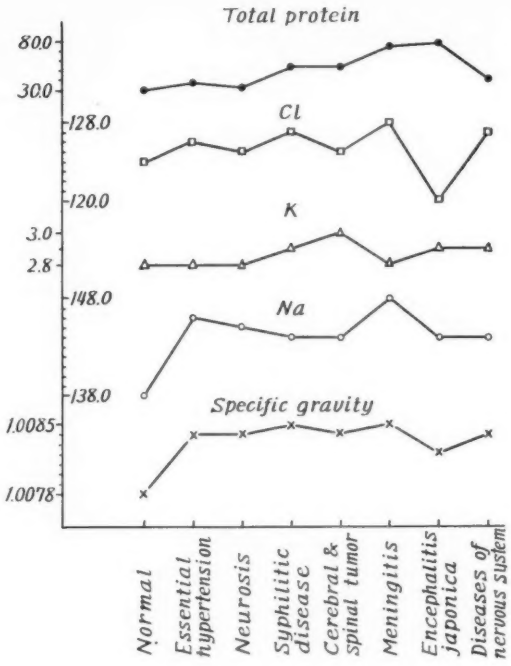
TABLE IV
Blockade of Ganglion Stellatum

Patient Name	Sex	Age	Diagnosis		Specific gravity	Na mEq/L	K mEq/L	Cl mEq/L	Total protein mg/dl
1) C.E.	♂	35	Neurosis	Before	1.0081	130.0	2.7	119.8	11
				After	1.0084	148.5	2.7	144.4	47
2) H.T.	♀	29	Neurosis	Before	1.0080	140.0	3.0	125.5	31
				After	1.0083	145.0	2.7	127.5	27
3) R.S.	♂	35	Mogigraphia	Before	1.0084	154.0	2.7	151.0	30
				After	1.0081	149.5	2.7	125.9	46
4) A.S.	♂	21	Paramyoclonus multiplex	Before	1.0085	135.5	3.2	117.9	31
				After	1.0087	151.5	2.7	127.7	46
5) K.S.	♂	36	Neurosis	Before	1.0084	144.5	2.7	125.2	32
				After	1.0086	145.5	2.8	130.6	36

Helmsworth[16], Shaw & Holley[17], Cooper, Lechner & Bellet[18] and Hinohara[19] have reported on their studies, but the numbers of the kinds or classes of the diseases they have treated are regrettably too small to be cited in comparison with my data.

The values of the specific gravity, the contents of electrolytes and total protein of spinal fluid obtained in my study may be illustrated as shown in Fig. 1, which shows that the curves plotting the values are all approximately similar in form, suggesting that these values stand in close mutual interrelation. It is also seen that in the non-normal cases showing specific gravity exceeding 1.0080, the contents of Na, Cl and total protein also show proportionate high values, and in the cases of considerably subnormal Cl contents, such as of encephalitis japonica, the specific gravity is relatively

Fig. 1



low, even when the Na and total protein is not lower than in the other groups. Furthermore, in the cases of diseases not included in the class of organic neural disturbance and showing no increase of the total protein content, as in the neurotic group, it must be especially noted that the specific gravity is rather high. In such samples of spinal fluid, the electrolyte content is always found supernormally heightened, too.

Of course the change in the specific gravity of the spinal fluid is not produced by a change in the contents of electrolytes and total protein alone, but also by many a delicate factor working in the complicated mechanism of secretion and absorption of other organic and inorganic matters and cellular constituents, but we can infer from the results above at least that there exists a close interrelation between the changes of the specific gravity and of the electrolyte and total protein content, and that these sets of values tend to show parallelism in their changes.

There is scarcely any study reported on the change of the specific gravity and the electrolyte content of the spinal fluid following alterative therapy, but as it is known that the serum content of electrolytes, especially

of chlorine, slightly and slowly rises in the first 4–5 hours after electric shock, it may be readily accepted as natural that my results revealed that the electrolyte contents and consequently the specific gravity are heightened.

It was, however, impossible to follow up the change of the contents by time as in blood, since the puncture for extracting spinal fluid cannot be repeated so freely.

Summary

1. The specific gravity of the spinal fluid remained always below 1.008 in normal subjects, but in the 7 groups of patients of neural diseases, i.e. of hypertensives, of neurotics, of syphilitic diseases, of cerebrospinal tumors, of meningitis, of encephalitis japonica and of other miscellaneous neural diseases, the individual values were higher than 1.008 except in a very few cases and the average values by groups also ranged between the supernormal levels of 1.0082 and 1.0085.

2. Of the contents of electrolytes in spinal fluid, Na was always higher in the diseased groups than in normal subjects, the deviation running up from 5.8 mEq/L to the height of 10.4 mEq/L. K showed little change by disease groups, the deviation remaining within 0.2 mEq/L at the highest. In comparison with the normal subject, the diseased cases showed Cl values higher by 0.8–4.0 mEq/L, except the encephalitis japonica cases, who showed values below normal by 4.2 mEq/L.

3. From such anomalies in the specific gravity and the contents of electrolytes and total protein in the cases of neural diseases, we can infer that the increase and decrease in these values run parallel to each other.

4. Following alterative therapies, the specific gravity and the contents of electrolytes, especially, of Na and Cl, of the spinal fluid were found risen above the pretreatment levels.

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Biochemical Studies on Carbohydrates

CCIX. Analysis of Mutarotation of N-Glycosides

Third Paper

Acetylation of Aniline-N-α- and -β-lactoside

By

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(Received for publication, October 25, 1957)

In a previous article, one[1] of the writers informed of the two anomeric aniline-N-lactosides. On full acetylation those isomers gave similarly analyzing products, which both melt at 155°C (Mixed melting point test showed no depression) and mutarotate from −40° (extraporated) to +23° (equilibrium) in 1 *N* acetic acid in methanol, proving to be heptaacetyl-aniline-N-β-lactoside.

As already mentioned[1], the two aniline-N-lactosides exhibit the same ultraviolet spectral absorption resembling that of aniline but with the maximum shifted 5 mμ toward the longer wave-lengths and heightened due to batho- and hyperchromic effect of the glycosidic sugar. The same held in the case of the spectrum of the present acetyl derivative, and no change of absorption occurred for two hours after dissolution of the substance, notwithstanding that 1.0 *N* acetic acid in methanol, in which the compound shows mutarotation, was employed as a solvent. This is an evidence for the fact that the mutarotation above is not ascribed to hydrolysis but to anomerization.

Experimental

Acetylation of aniline-N-β-lactoside. 1 g. of aniline-N-β-lactoside[1] was added to a mixture of 10 cc. of pyridine and 5 cc. of acetic anhydride and at first placed in an ice-chest (0°C) for 4 days with frequent shaking and afterwards stood at room temperature (15°C) for one day. The yellowish solution was precipitated with 250 cc. of petroleum ether. The deposit was kneaded in 5 cc. of abs. ethanol and after addition of 60 cc. of petroleum ether, filtered off. Yield here 1.2 g. (71% of theory). F.P. 135–140°C. $[\alpha]_D^{16}$ (chloroform ; c. 1.8) was −35.5°.

1.1 g. of the crude product was dissolved in 10 cc. of chloroform, 30 cc. of *n*-hexane was added, and the mixture was stood in a refrigerator (0°C) overnight. 0.99 g. of crystals in prisms was given. Recrystallized three times from dry methanol, the product showed a constant melting point of 155°C. Yield 0.62 g.

Its $[\alpha]_D^{15}$ was $-41.0°$ in chloroform (c. 1.8), $-42.3°$ in dry methanol (c. 2.1), $-48.7°$ in dry pyridine (c. 2.62) and $-47.5°$ in pyridine containing 10% water (c. 2.12). In these solvents it showed no mutarotation. N 1.96% ; acetyl 42.4%. Calcd. for $C_{32}O_{17}H_{41}$: N 1.96%, acetyl 42.2%.

Acetylation of aniline-N-α-lactoside. 0.8 g. of aniline-N-α-lactoside was acetylated as the β-isomer was. The crude product amounted to 1.1 g. (81% of theory). F.P. 146–148°C. $[\alpha]_D^{14}$ (chloroform ; c. 1.76) $-33.8°$. It was purified also as above. The crystals given weighed 0.35 g. F.P. 155°C. $[\alpha]_D^{14}$ (chloroform ; c. 1.85) $-42.0°$. $[\alpha]_D^{15}$ (dry pyridine ; c. 2.08) $-46.8°$. No mutarotation occurred in these solvents. N 1.94% ; acetyl 41.8%.

The two fully acetylated products showed no depression of melting point when mixed.

Mutarotation. The acetyl derivative of aniline-N-β- and -α-lactoside mutarotated along the same course in 1 *N* acetic acid in methanol. Temperature of test 16°C. See Table I and Fig. 1.

TABLE I

Mutarotation of the Acetylated Product of Aniline-N-α-lactoside in 1 *N* Acetic Acid in Methanol

c. 2.49. Temp. 16°C.

Time after dissolution (min.)	$[\alpha]_D$ (°)
5	−20.5
10	−14.1
26	− 0.6
36	+ 4.2
60	+11.8
120	+18.2
270	+19.5
60×24×2	+22.7

Spectral absorption. 55.9 mg. of heptaacetyl-aniline-N-β-lactoside was dissolved in 2 cc. of 1 *N* acetic acid in methanol, and immediately and after standing at 16°C for two hours, 0.1 cc. of the solution was made up to 100 cc. with dry methanol, followed by measurement of extinctions at various wave-lengths in the ultraviolet region. No alteration of absorption took place at any wave-length within the two hours of standing. For the reference the spectral absorption of aniline and pentaacetylgalactose was measured. In this case, 0.337 mg. of aniline and 10.1 mg. of pentaacetylgalactose were dissolved each in 0.1 cc. of 1 *N* acetic acid in methanol and made up to 100 cc. with dry methanol. See Table II.

SUMMARY

The two anomers of aniline-N-lactoside give the same acetyl product

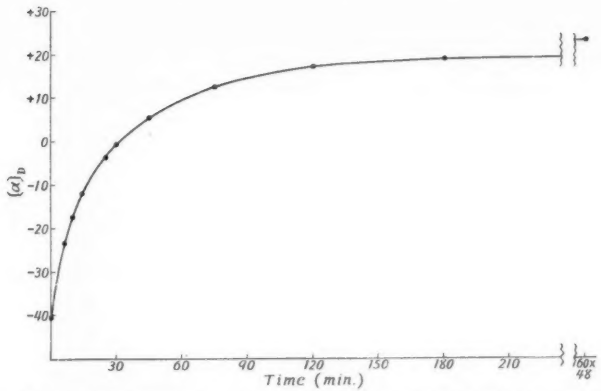

Fig. 1. Mutarotation in 1 *N* acetic acid in methanol of the acetylated product of aniline-N-β-lactoside. c.=2.80. Temp. 16°C.

TABLE II

Extinctions (−log T) of Heptaacetyl-aniline-N-β-lactoside, Aniline and Pentaacetyl-galactose

Wave-length (mμ)	−log T			
	Heptaacetyl-aniline-N-β-lactoside		Aniline	Pentaacetyl-galactose
	Immediately after dissolution in the *N* CH_3COOH	After 2 hrs.		
220	0.325	0.318	0.162	0.105
225	0.412		0.230	
230	0.540		0.278	0.070
235	0.668		0.276	
240	0.678	0.660	0.234	0.011
245	0.483		0.171	
250	0.181		0.103	0.004
255	0.049		0.052	
260	0.037	0.037	0.031	0.002
265	0.042		0.029	
270	0.061		0.034	0.001
275	0.075		0.043	
280	0.089		0.052	0.000
285	0.090	0.091	0.049	
290	0.076		0.046	0.000
295	0.054		0.040	
300	0.024	0.024	0.029	0.000
310	0.006		0.005	
320	0.001		0.001	

which has been established to be heptaacetyl-aniline-N-β-lactoside.

Through the Grant Committee for Scientific Researches the Ministry of Education gave a grant in aid to us, which is gratefully acknowledged.

H. Masamune.

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Biochemical Studies on Carbohydrates

CCX. A Systematic Fractionation of Pig Gastric Mucosa with Respect to Molisch-positive Hexosamine-containing Components

By

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In pig gastric mucosa has been mentioned the presence of various mucopolysaccharides and mucoproteins such as Group AO mucopolysaccharide, mucoitin[1], gastroitinsulfuric acid[2], chondroitinsulfuric acid[3], heparin[4], a glacial acetic acid-soluble glucidamin[5], a glucidamin insoluble in water at pH 2.0[6] and Castle's intrinsic factor.

To prepare Castle's intrinsic factor, Williams *et al.*[7] applied tryptic digestion which probably decomposed the factor partially. Latner *et al.*[8] and Cresseri[9] separated it by preparative electrophoresis and other operations and by paper electrophoresis with subsequent phenol-treatment and fractional alcoholic precipitation respectively. And the preparation of Cresseri was almost devoid of group activity. Although the preparations obtained by these investigators differed from each other in composition remarkably (The preparation of Latner *et al.* contained 10% N, 6.5% hexosamine, 2.2% L-fucose and 13% reducing sugars as glucose, and that of Cresseri 8.27% N, 17.1% hexosamine and 18.3% reducing sugars), it is quite definitely revealed that the intrinsic factor of Castle is a glucidamin. Apart from this, all the components above of pig gastric mucosa, the group mucopolysaccharide[10] excepted, have not been as yet isolated in a state perfectly homogeneous electrophoretically, that is, in a state warranted to be homogeneous after electrophoresis both at alkaline as well as acid side.

The present account deals with systematic fractionation in a mildest way of the Molisch-positive hexosamine-containing components of the mucosa. Substances I–IV, of which three were mucopolysaccharides and one was a glucidamin, were separated in a pure state in the mentioned aspect.

Experimental

Fractionation Procedure

Fresh pig stomach mucosa freed from mucus mechanically was ground in a masticator, and after drying in a Faust apparatus, shaken with 8 and 5 volumes

of water at 5°C in succession, each extraction taking 24 hours. The operation was processed without addition of a preservative to avoid denaturation of proteins, but no putrifaction occurred. 200 g. (9 stomachs) and 132 g. (6 stomachs) of the dry material were employed, and in each case, the solutions were united (Fluids I and II).

Fractionation of the Fluids was carried out as will be described in full on the run with Fluid I.

The fluid (2 *l.*) was acidified to pH 4.2 with 30% acetic acid and stood in an ice-chest overnight, followed by centrifugation. The centrifugate will be referred to as Fr. 1. The very turbid supernatant was added to with 99% alcohol to 15% and stood again in the ice-chest overnight. The deposit given was also centrifuged off—Fr. 2. And the supernatant was further fractioned by repetitions of the addition of alcohol and following treatment above. The successive fractions separated at 30, 45 and 60% concentrations of alcohol are named Fr. 3, 4 and 5 respectively. The final centrifuged supernatant was distilled to 1/3 the volume in vacuo to expel most of the alcohol, dialyzed for 2 days against running water and distilled to a syrup which was precipitated with excessive alcohol containing sodium acetate—Fr. 6. As shown in Table I, all

TABLE I

Yield and Composition of Frs. 1–6

Analytical figures express percentages.

		Fr.					
		1	2	3	4	5	6
Yield (g.)		17.8	2.0	1.0	0.9	10.2	6.6
Analysis	N*	12.6	12.2	12.0	11.5	9.0	10.3
	Hexosamines as glucosamine†	2.4	1.8	1.6	2.3	10.6	7.8
	L-Fucose‡	0.05	0.1	0.1	0.3	2.7	1.9

* Micro Kjeldahl. † Masamune and Yosizawa[13], ‡ Masamune and Sakamoto[15].

of Frs. 1–4 were found poor in carbohydrate. The balances of Fr. 5 and 6 which contained no small amount of hexosamine were taken up together in 500 cc. of water, and the turbid, viscid solution was added to with dilute adetic acid to pH 4.2 at first and then with 99% alcohol to 40%, and after standing overnight in the ice-chest, centrifuged. The centrifugate, which weighed 1.2 g. when dry, was discarded. To the supernatant was added additional alcohol to 50%, whereby a voluminous, fibrous precipitate (Fr. 7) occurred, giving a non-viscous mother fluid. On further addition of alcohol to 60%, the mother fluid gave 1.1 g. of a precipitate (Fr. 8), and from the mother fluid of this precipitate was recovered 6.0 g. of a substance (Fr. 9) by condensation and addition of a large volume of alcohol containing sodium acetate.

Fr. 7 was taken up in 500 cc. of water and acidified to pH 4.2 with acetic

acid, the precipitate occurring on addition of alcohol to 42.5% (2.4 g.) was rejected, and the substance that precipitated on raising the alcohol concentration till 47.5% was set aside (Crude I, yield 2.5 g.).

Fr. 8 was dissolved in 100 cc. of water and the non-viscous, clear solution was adjusted to pH 4.2 as above. And the precipitate on addition of 99% alcohol to 50% (0.5 g.) was removed, and that at the concentration range of alcohol from 50 to 60% (Crude II, yield 0.5 g.) was taken.

Fr. 9 was dissolved in 200 cc. of water and acidified to pH 4.2 as above, and the precipitate that occurred on addition of alcohol to 60% was centrifuged off. The whole substance in the supernatant was recovered similar to above and taken up in 200 cc. of water, followed by adjustment of the acidity to the same pH also as above. The solution was then saturated with $(NH_4)_2SO_4$. The precipitate was separated after standing in the ice-chest overnight, and after 3 reprecipitations, dialyzed against running water for 3 days and electrodialyzed in a Pauli apparatus for 3 days. The deposits after these operations were thrown away, and the solution was condensed and precipitated with sodium acetate-containing alcohol—Crude III (0.6 g.). The substance which was not carried down by $(NH_4)_2SO_4$ was also recovered in a similar manner after 5 days' dialysis and 3 days' electrodialysis—Crude IV (0.5 g.).

Purification of Crude I. 2.5 g. of Crude I was shaken with 100 cc. of 90% phenol (a mixture of 9 parts of phenol and 1 part of water by volume) overnight. The insoluble part was centrifuged off, which weighed 0.2 g. when dry. The supernatant was added to with a mixture of equal volumes of abs. alcohol and of 90% phenol to alcohol concentration of 10%, whereby a gelatinous precipitate occurred. The precipitate was centrifuged, washed with alcohol and with ether and dried (1.0 g.). It was again taken up in 90% phenol, precipitated by addition of alcohol to 10% and subjected to a reprecipitation. Yield here was 0.65 g. It was next dissolved in 50 cc. of water. To the solution was added crystalline barium acetate to 3%, and the solution was adjusted to pH 7.0 with dilute acetic acid, added to with alcohol to 45% and stood overnight in the ice-chest. The deposit was rejected after centrifugation, and the supernatant was further added to with alcohol to 50% and again centrifuged after standing in the ice-chest. The centrifugate here was dissolved in water and decomposed by addition of crystalline sodium sulfate. The centrifuged supernatant was dialyzed for 3 days, carried down with excessive alcohol containing sodium acetate and washed with abs. alcohol, followed by drying in vacuo over $CaCl_2$—Prep. I. Crop 0.4 g. (0.2% of the dry tissue material).

Purification of Crude II. 0.5 g. of Crude II was dissolved in 25 cc. of 90% phenol, and alcohol was added to 10%. The part precipitated was discarded, and that precipitated on increasing alcohol to 15% was separated and subjected twice more to similar fractionation. Yield here 0.2 g. It was dissolved in 20 cc. of water, and after addition of crystalline barium acetate to 3% and following adjustment of the pH to 7.0, precipitated with 20 cc. of abs. alcohol. The centrifuged supernatant was added to with additional alcohol to 60%, and the deposit was dissolved in water, decomposed with sodium sulfate and precipitated with sodium acetate-containing alcohol. 0.17 g. of substance was given. This

fraction was dissolved in $M/5$ acetate buffer of pH 4.2 and shaken with four changes of a chloroform-amylalcohol mixture (5:1 by volume) to free from coagulable proteins. The solution was further liberated from inorganic salts by 3 days' dialysis and 3 days' electrodialysis, precipitated perfectly with alcohol

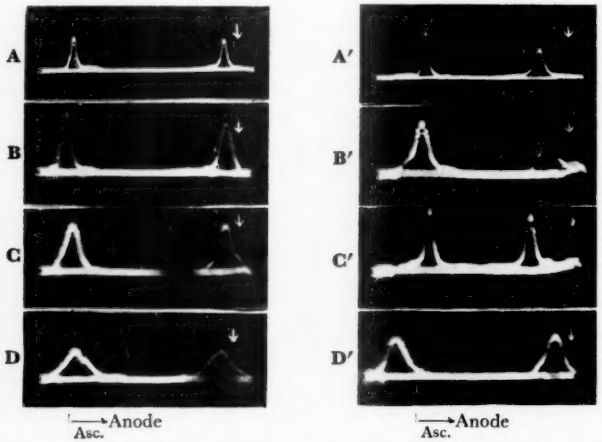

Fig. 1. Electrophoretic patterns (2/3×) of 1% solutions of Prep. **I, II, III** and IV in acetate buffer of pH 4.7 and I 0.2 (**A**, **B**, **C** and **D** respectively) and phosphate buffer of pH 7.7 and I 0.2 (**A′**, **B′**, **C′** and **D′** respectively); current 10 mA; temperature 15°C. Exposure 60 minutes after starting current. Ascending limbs to the left. ↓ signifies the starting boundary.

Mobility:

Prep. I at pH 4.7 $\dfrac{(-0.15)\times 0.0063\times 0.3}{3600\times 0.61} = -0.8\times 10^{-5}$ cm^2·volt^{-1} sec^{-1}

at pH 7.7 $\dfrac{(-0.6)\times 0.0068\times 0.3}{3600\times 0.01} = -3.4$ "

Prep. II at pH 4.7 $\dfrac{(-0.15)\times 0.0063\times 0.3}{3600\times 0.01} = -0.8$ "

at pH 7.7 $\dfrac{(-0.35)\times 0.0068\times 0.3}{3600\times 0.01} = -2.3$ "

Prep. III at pH 4.7 $\dfrac{(-0.2)\times 0.0063\times 0.3}{3600\times 0.01} = -1.1$ "

at pH 7.7 $\dfrac{(-0.85)\times 0.0068\times 0.3}{3600\times 0.01} = -4.8$ "

Prep. IV at pH 4.7 $\dfrac{(-0.12)\times 0.0063\times 0.3}{3600\times 0.01} = -0.6$ "

at pH 7.7 $\dfrac{(-0.5)\times 0.0068\times 0.3}{3600\times 0.01} = -2.9$ "

containing sodium acetate and washed with alcohol as above—Prep. II. The product dried weighed 97 mg. (0.05% of the dry tissue material started with).

Purification of Crude III. 0.6 g. of Crude III was dissolved in 60 cc.

Fig. 2

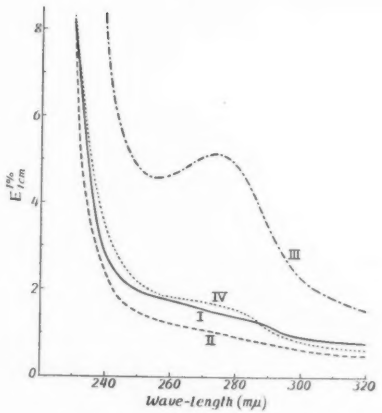

Fig. 3

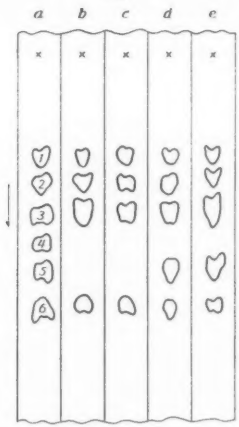

Fig. 2. Ultraviolet absorption spectra of 0.06% solutions in water of Prep. I, II, III and IV.

Fig. 3. Sugar chromatograms (1/10×) of the hydrolysates of Prep. I (*a*), II (*b*), III (*c*) and IV (*d*) developed by irrigation with the mixture of butanol, pyridine and water. Temperature and duration of test, 23°±1°C and 24 hrs. respectively. *e*: reference run, *1*: galactosamine, *2*: glucosamine, *3*: galactose, *4*: glucose, *5*: mannose, *6*: L-fucose.

of water, half-saturated with $(NH_4)_2SO_4$ (solid) and centrifuged after standing overnight at room temperature. The supernatant was added to again with solid $(NH_4)_2SO_4$ till 3/4 saturation and stood overnight. The centrifugate here was also dissolved in water and dialyzed for 5 days against running water. The solution was then distilled in vacuo to 50 cc., crystalline barium acetate was added to 3%, and after adjustment to pH 7.0, the mixture was added to with alcohol to 60%, followed by standing overnight in the ice-chest. A small precipitate occurring was rejected, and the alcohol content of the clear mother fluid was increased to 80%. The precipitate was separated on the next day, and after dissolving in water and eliminating barium with sodium sulfate, electrodialyzed. A trace of an occurring tiny precipitate was rejected. The solution was then distilled in vacuo to a syrup, alcohol containing sodium acetate was added, and the deposit was washed with abs. alcohol. The product weighed 117 mg. when dry (0.06% of the dry tissue material)—Prep. III.

Purification of Crude IV. 0.5 g. of Crude IV was dissolved in 50 cc. of 90% phenol, and the centrifuged supernatant (0.1 g. was the insoluble part) was precipitated by addition of alcohol to 30%. The substance given (0.35 g.) was reprecipitated in a similar manner and then precipitated three times from 90% phenol by addition of alcohol to 20%. The amount of substance was reduced to 180 mg. Next it was taken up in water, added to with barium acetate to 3% and fractionated at pH 7.0 with alcohol. The part which pre-

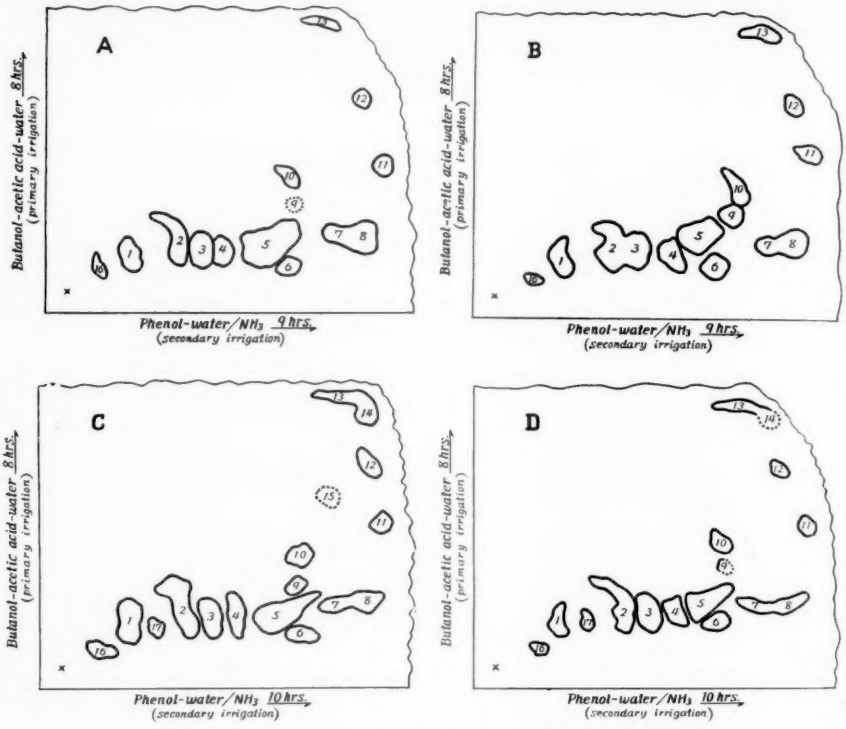

Fig. 4. Amino acid chromatograms (3/14×) of the hydrolysates of Prep. I (**A**), II (**B**), III (**C**) and IV (**D**). Temperature of test 23°±1°C. *1*: aspartic acid, *2*: glutamic acid, *3*: serine, *4*: glycine, *5*: hexosamine, *6*: lysine, *7*: histidine, *8*: arginine, *9*: threonine, *10*: alanine, *11*: proline, *12*: valine and/or methionine, *13*: leucine and/or isoleucine, *14*: phenylalanine, *15*: tyrosine, *16*: a probable peptide, *17*: an unknown spot.

cipitated at the concentration range of alcohol from 60 to 80% was dissolved in water and decomposed with sodium sulfate, and the centrifuged supernatant was electrodialyzed, condensed, precipitated with alcohol containing sodium acetate and washed with abs. alcohol. 88 mg. (0.04% of the starting tissue material) was the substance given—Prep. IV.

From Fluid II were obtained Preps. I′–IV′ corresponding to Preps. I–IV above and weighing 240, 60, 53 and 50 mg. (0.18, 0.05, 0.04 and 0.04% of the starting dry tissue material) respectively.

To add, of Frs. 1–4, which were set aside because of tiny content of hexosamine, Fr. 1 containing the largest amount of hexosamine was subdivided by the aid of 90% phenol, barium acetate and increasing volumes of alcohol. The subdivision richest in hexosamine analyzed and rotated D-light similar to Prep. I,

suggesting that Fr. 1 was a mixture containing a part of the latter component.

Finally the tissue residue freed from water-soluble components was subjected to extraction with 10% $CaCl_2$ and then to that with 0.5 *N* NaOH. The extracts were almost Molisch negative, whereas the one by 10% $CaCl_2$ gave distinct Goldschmiedt reaction.

Properties of the Preparations

Electrophoresis. All of Preps. I–IV showed one symmetrical boundary after electrophoresis at pH 4.7 as well as at pH 7.7 as shown in Fig. 1, although in III–IV the boundary spreading proved reversible.

Ultraviolet absorption. The ultraviolet absorption of Preps. I–IV was measured in a modified Beckman spectrophotometer (Hitachi EPB-V type). Prep. I, II and IV showed no particular absorption contrary to III which absorbed distinctly the light at the wave-length of 275 mμ due to cyclic amino acids. See Fig. 2.

Solubility. All of Preps. I–IV dissolved readily in water, and among others Prep. I gave a viscous solution, from which it was carried down as a fibrous precipitate with alcohol. Not only III but also I was precipitated from water by saturation with $(NH_4)_2SO_4$ and with NA_2SO_4. Prep. I also precipitated from water when its solution was electrodialyzed. In glacial acetic acid Prep. I, II and IV were found insoluble, but III readily soluble. To add, Prep. III formed no gel, when its solution in 0.2 *N* acetate buffer pH 4.0 was shaken with the chloroform-amylalcohol mixture.

Test-tube tests. Among 0.3% solutions of Preps. I–IV, only that of III gave the positive biuret reaction. Goldschmiedt's and Neuberg-Saneyoshi's tests resulted in negative in all of those preparations. Prep. I and II proved to contain hydrolyzable sulfur, and III and IV were slightly and faintly Hopkins-Cole (test for tryptophan) positive respectively.

Identification of the sugar components by paper partition chromatography. 15 mg. of substance was hydrolyzed with 1.5 cc. of 1 *N* H_2SO_4 in a sealed tube by heating in boiling water for 5 hours. The hydrolysate was freed from sulfuric acid with baryta, distilled in vacuo to dryness and dissolved in 0.3 cc. of water. A sliver of Toyo Roshi filter paper No. 2 was spotted with a 0.025 cc. portion of the solution and irrigated in the descending way with a butanol-pyridine-water (5:3:2 by volume) mixture. Indicator aniline hydrogen phthalate reagent of Partridge. As shown in Fig. 3, all the hydrolysates of the preparations gave spots at the positions corresponding to galactosamine, glucosamine, L-fucose and galactose, and III and IV gave a strong spot of mannose besides. No spot of pentose appeared in any of the preparations. A chromatogram was also developed by replacing a *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1 by volume) mixture for the irrigation solvent above, but the result was the same.

Identification of the amino acid components by paper partition chromatography. 6 mg. of substance was heated with 0.6 cc. of 6 *N* HCl in a sealed tube at 100°C for 24 hours, and the hydrolysate was freed from the hydrochloric acid as far as possible by evaporation in a vacuum desiccator containing solid caustic soda

with repeated additions of water, as used to in this Institute, and the final evaporated residue was taken up in 0.1 cc. of water. 0.03 cc. of the solution was sampled on a 30×30 cm. square of Toyo Roshi filter paper No. 2. Irrigation was processed by the ordinary way, employing a butanol-acetic acid-water (4:1:2 by volume) mixture and 80% phenol/NH_3 for the primary and secondary irrigations respectively. Indicator 0.1% ninhydrin in butanol saturated with water. All the preparations proved to contain aspartic and glutamic acids, serine, glycine, lysine, histidine, arginine, threonine, alanine, proline, valine and/or methionine and leucine and/or isoleucine in common. Prep. III gave spots of tyrosine (weak) and phenylalanine, and Prep. IV that of phenylalanine (weak) in addition. Moreover, the chromatograms of the substances showed a spot of a probable peptide or a spot of a probable peptide and an unknown ninhydrin positive spot. See Fig. 4.

Quantitative composition. The preparations were dried to a constant weight at 105°C over $CaCl_2$ before analysis. In Table II are embodied the analytical figures together with optical rotations.

Blood group potency. By the glass plate method described by Masamune *et al.*[11] was given the result in Table III. Prep. I and II were strongly group A- and weakly group O-active. III and IV possessed also the same group activities but were very weak, suggesting minute contamination with I or II.

TABLE II

Composition and Optical Rotation of the Preparations

Analytical results are expressed in per cent and rotations in degrees.

Analyses and rotation	Preparation							
	I	I′	II	II′	III	III′	IV	IV′
N*	5.6	5.6	6.0	5.9	9.0	9.3	4.9	4.4
Hexosamines as glucosamine†	35.2	33.1	35.2	33.8	14.0	13.5	20.8	22.0
Galactose‡	20.8	22.0	20.4	21.6	6.7	5.7	15.0	15.8
Mannose‡					3.2	4.6	12.7	13.2
L-Fucose§	8.5	8.0	4.6	4.0	2.7	2.6	5.6	5.7
Sialic adid//		4.9		3.6		19.1		19.5
Ash¶	2.1	3.0	0.8	1.3	1.0	1.1	1.2	0.6
$[\alpha]_D^{20}$ in water	+13	+11	+24	+29	−12	−11	−24	−21

* Micro Kjeldahl. † Masamune and Yosizawa[13], ‡ Masamune and Sakamoto[14], § Masamune and Sakamoto[15], // Prof. K. Turumi of Fukushima Medical School kindly analyzed it by the direct Ehrlich reaction according to Werner and Odin[16], employing sialic acid isolated by him as the standard. ¶ Pregl method without use of H_2SO_4.

Discussion

As described above, four Molisch positive, Goldschmiedt-negative, hexosamine-containing, high-molecular components have been separated

TABLE III

Anti-hemagglutinative Potency of the Preparations

The sera were diluted to titre 16 before use. Goat: normal anti-O goat serum. Anti-OS saliva: anti-OS saliva chicken serum. − : no hemagglutination. + : slight hemaggl. ++ : marked hemaggl. # : strong hemaggl.

Preparation	Red cells	Serum	Dilution of the substances (1:)										
			10^2	10^3	10^4	10^5	5×10^5	10^6	5×10^6	10^7	5×10^7	10^8	5×10^8
I	A	α	−	−	−	−	−	−	−	−	−	−	+
	B	β	++	++	#	#	#	#	#	#	#	#	#
	O	Goat	−	−	−	++	#	#	#	#	#	#	#
	O	Anti-OS saliva	−	−	−	−	+	++	#	#	#	#	#
II	A	α	−	−	−	−	−	−	+	+	++	++	++
	B	β	++	++	#	#	#	#	#	#	#	#	#
	O	Goat	−	−	#	#	#	#	#	#	#	#	#
	O	Anti-OS saliva	−	−	+	#	#	#	#	#	#	#	#
III	A	α	−	+	++	#	#	#	#	#	#	#	#
	B	β	++	++	#	#	#	#	#	#	#	#	#
	O	Goat	−	++	#	#	#	#	#	#	#	#	#
	O	Anti-OS saliva	−	+	#	#	#	#	#	#	#	#	#
IV	A	α	−	−	+	++	#	#	#	#	#	#	#
	B	β	++	++	#	#	#	#	#	#	#	#	#
	O	Goat	−	−	++	#	#	#	#	#	#	#	#
	O	Anti-OS saliva	−	+	++	#	#	#	#	#	#	#	#

from pig gastric mucosa. One of them (III) is a glucidamin and the others (I, II and IV) mucopolysaccharides in Masamune's terminology[12]. The analytical figures of III resemble those of the glacial acetic acid-soluble, group-active glucidamin of Satoh[5]. The writers' glucidamin dissolves also in glacial acetic acid but is group-inactive, their faint group activity being regarded as due to slight contamination with I or II. Satoh did not examine his preparation with respect to its homogeneity, and hence that preparation was a probable mixture containing I or II. Castle's intrinsic factor looks to be a group-inactive glucidamin judging from the findings of the predecessors already mentioned so that III, which is the sole and group-inactive glucidamin in pig stomach mucosa, is assumed to be that factor of Castle.

Regarding the three mucopolysaccharides, the group-inactive IV markedly differs from I and II, because it is levo- but not dextro-rotatory and contains mannose as a hexose besides galactose and less hexosamine and more sialic acid. Its biological significance awaits a study. I is particularly characterized in giving a very viscous solution and possessing an extremely high group A potency. This mucopolysaccharide generally resembles II in composition, but the L-fucose content of it approximates to

twice the corresponding content of II. Furthermore, I is dextrorotatory only half as much as II, and the latter gives a non-viscous solution and is not precipitated with $(NH_4)_2SO_4$ and Na_2SO_4 contrary to the former. II is not accepted to be a degradation product of I, judging from the mild procedure of fractionation of the tissue extract.

Summary

1. Pig stomach mucosa was systematically fractioned in a mildest way to isolate all the hexosamine-containing, Molisch-positive components.

2. One glucidamin and three mucopolysaccharides of which two are group-active were obtained.

3. The glucidamin is supposed to be Castle's intrinsic factor.

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Retinal Induction in the Light-Adapted Human Eye

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Introduction

Motokawa found a remarkable phenomenon that the electrical excitability of the eye is changed by light. In his investigation[1] the electrical excitability was pursued for several seconds after preillumination with various colored lights; after a refractory period, the excitability becomes supernormal and then returns to the previous level in several seconds. The time required for reaching a maximum of electrical excitability is related neither to the intensity nor to the duration of preillumination.

The crest time is characteristic of the stimulated receptor and about 1, 1.5, 2 and 3 sec. for red, yellow, green, and blue lights respectively.

Motokawa, moreover, applied the method mentioned above to the problem of contrast, and found a physiological correlate of contrast, which was termed "retinal induction". Two kinds of retinal induction, direct and indirect, were distinguished; the former corresponding to successive contrast confined to a retinal area preilluminated, and the latter to simultaneous contrast found in the surrounding areas.

The experiments on retinal induction have been carried out in dark-dapted eyes.

In the present experiment, therefore, investigations were carried out in light-adapted eyes in order to make comparison with data obtained in different adaptation states of the eyes.

Experimental

Method

To control the degree of light adaptation, the illumination on a white screen standing vertically in front of the subject's eye was varied from 0.0004 lux to 30 lux. The distance between the screen and the eye was always 40 cm. In order to make the illumination on the screen uniform, a number of small lamps

were arranged on either sides of the face of the subject, in such a manner that the lights from these lamps did not directly reach the eyes, but illuminated the white screen in front of the subject.

The intensity of the light was controlled by means of neutral filters over the lamps. And in the middle of the screen there was a small opening which subtended 2° in visual angle. That opening was covered from behind by a cylindrical pipe of 20 cm. in length and 5 cm. in diameter. The surface at the bottom of the pipe consisted of opaque glass, and the eye could be exposed to the test light, colored or white coming from the opening. The opening of the screen could be covered by means of a cover which was as white as the screen. The intensity of the test light was always 100 lux at the bottom of the pipe.

The colored lights used were red (670 mμ), green (505 mμ) and blue (450 mμ) lights from respective interference-filters, and the white test light used was alway 8 lux in intensity.

The arrangements and procedure for electrical stimulation of the eye were the same as described in the preceding paper[2].

The experiments were performed after a preliminary light adaptation of about 10 minutes.

The constant current of 100 msec. was applied to the eye through a pair of silver electrodes 2×1.5 cm^2 in size with a cathode placed on the forehead slightly above the eyebrow and an anode on the homolateral temple of the subject. The contact of the electrodes with the skin was secured by electrode paste.

When the eye had been completely adapted to the light from the screen, the electrical excitability E_0 (the reciprocal of the electrical threshold) was determined. Then, the electrical excitability E at varying moments after two seconds exposure to the test light was determined. The effect of light was expressed quantitatively by a term ζ, which denoted a percentage increase in electrical sensitivity following an illumination ; $\zeta = 100\ (E-E_0)/E_0$. The retinal induction effect was determined by the difference between the ζ value for successive stimuli, colored and white, and the ζ value for the white test light alone, and the difference was denominated a contrast effect.

Results

Electrical excitability curves

The excitability curves for red, green and blue lights obtained under light adaptation of various intensities are illustrated in Fig. 1. As can be seen in this figure, the time course of electrical excitability is in shape almost the same as that of the dark-adapted eye. That is, when red, green and blue lights are used for pre-illumination, the peaks of the electrical excitability curves lie at 1, 2 and 3 seconds respectively. With increases in intensity of the adapting light from 0.04 lux to 30 lux, the magnitude or height of the curve decreases gradually. These experiments show definitely that the time course of electrical excitability is not affected by light adaptation, but that the magnitude is diminished by it.

The relation of the retinal induction and the interval between the inducing light and

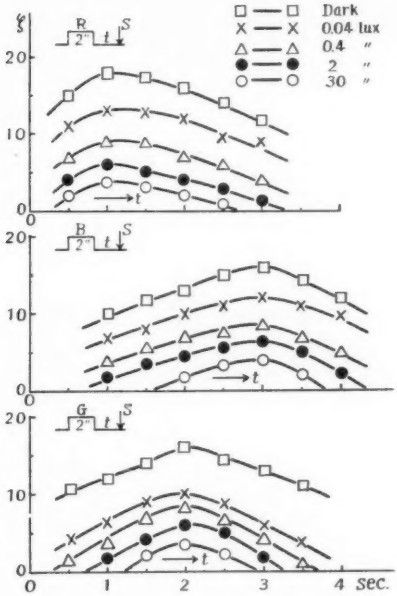

Fig. 1. Curve of electrical excitability of human retina. Ordinates: Values of percentage increases of electrical excitability caused by colored lights. Abscissae: Time in seconds after termination of light.

the white test light

The colored lights used for preillumination were red, green and blue lights from the interference-filters. The duration of preillumination was 2 seconds. The white test light was 8 lux in intensity and 2 seconds in duration. The interval between the colored light and the white test light was varied from 0 to 5 seconds. The electrical excitability was measured at various intervals from the end of the white test light. The experiments were carried out at an adaptation level of 0.004 lux.

A) Red light. The result obtained with red inducing light is shown in Fig. 2A. The broken curve connecting solid circles refers to a control experiment carried out in the dark-adapted state of the eye. The other curves were obtained from the light-adapted eye with varying intervals between the red and the white lights. The curve connecting empty circles represents that for the white test light alone.

As can be seen in this figure, the electrical excitability curve of the dark-adapted eye is higher than those of the light-adapted.

The magnitude decreases with increases of the interval, although the shape of the curve is little dependent on the interval.

Strictly speaking, some deformation occurs when the interval becomes

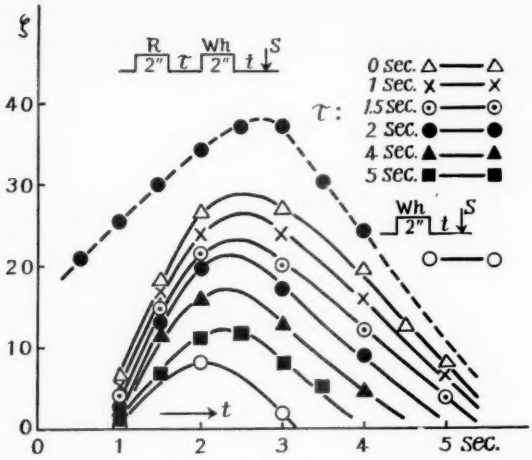

Fig. 2A. Curves of direct induction caused by red light. Ordinates: Percentage increases of electrical excitability above resting level. Abscissae: Time in sec. after end of preillumination. Procedure for experimentation is shown in insets where R, Wh, τ, S and t represent red light, white light, interval between R and Wh, an electrical stimulus and the interval between Wh and S respectively.

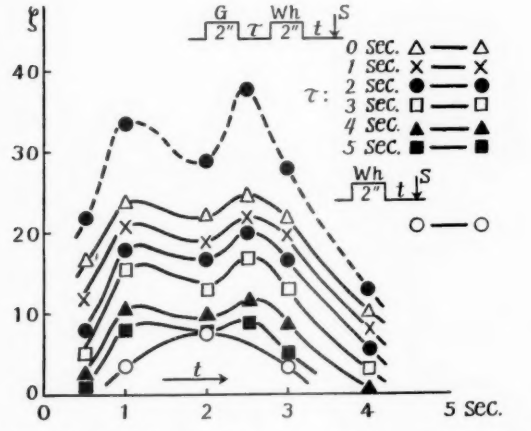

Fig. 2B. Curves of direct induction caused by green light (G). Other explanations as in A.

longer; ζ values are depressed more strongly towards the end of the ζ-time curve so that the maximum tends to shift to the left for longer intervals. This effect is related with the circumstance that in the light-adapted eye ζ decreases

with time, as will be mentioned later.

B) Green light. The broken curve connecting solid circles shown in Fig. 2B refers to that obtained from the dark-adapted eye. It has two peaks at 1 and 2.5 seconds, and this characterizes the direct induction caused by green light.

The same characteristic is retained in the curves obtained from the light-adapted eye. The magnitude decreases as the interval between the green light and the white test light is lengthened.

C) Blue light. The result is represented in Fig. 2 C. The broken curve is

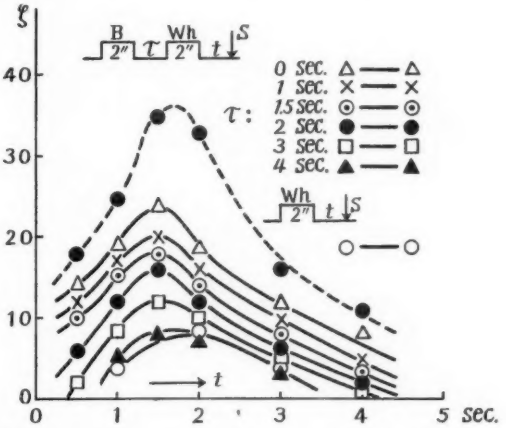

Fig. 2C. Curves of direct induction caused by blue light (B). Other explanations as in A.

a control curve. All the curves have a maximum at about 1.5 sec. The magnitude decreases with increasing intervals separating the inducing and the test lights. When this is 4 sec. the curve obtained almost coincides with the curve obtained with the white test light alone (cf. the curve marked by solid triangles with that marked by empty circles). This fact indicates that direct retinal induction disappears in about 4 seconds at the retina adapted to 0.004 lux.

Decay of the contrast effect of the light-adapted eye

The contrast effect caused by the red light was represented by the difference between the ζ-values at 2 seconds of each curve and that for the white test light alone in Fig. 2 A.

In Fig. 3 the contrast effect was plotted against the interval between the red and the white lights. As can be seen in this figure, the contrast effect declines linearly with time, and disappears in about 6 seconds.

The contrast effects caused by the green light and by the blue light were defined by similar differences in ζ at 1 and 1.5 seconds respectively. The contrast effects by the green and by the blue light decline in the same manner as above (see curves connecting solid circles and solid triangles). Such a linear

decline of the contrast effect is seen only under light adaptation.

In the completely dark-adapted state of the retina, the contrast effect remains unchanged for about 20 seconds from termination of the inducing colored light. It takes about 40 seconds for complete vanishment of the contrast effect in the dark-adapted retina. In marked contrast with this, the contrast effect disappears in 4–6 seconds in the retina adapted to white illumination of 0.004 lux.

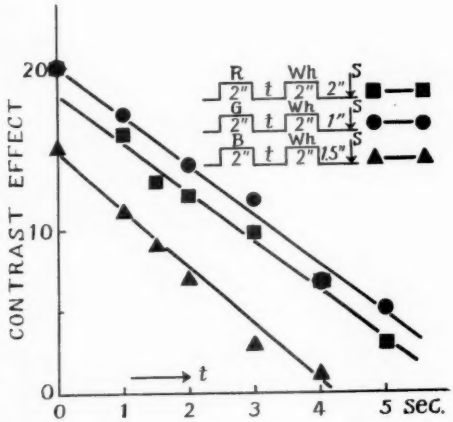

Fig. 3. Temporal decline of direct retinal induction in light-adapted retina. Ordinates: Contrast effect. Abscissae: Time interval between inducing light and white test light.

One may suppose that the adapting white light destroys the contrast effect. If this interpretation were correct, the decay of the contrast effect would be facilitated by a strong white light interposed between the inducing colored light and the subsequent white test light.

In reality, however, such an interposed white light had no effect upon the decline of the contrast effect, as is shown in Fig. 4. In this experiment a white light stimulus of 32 lux in intensity and 1 second in duration was given in succession to the red inducing light. It was shown by Nakagawa & Kohata[5] that no effect could be seen upon the contrast effect of the dark-adapted retina when a white light stimulus as strong as 1,000–10,000 lux was interposed between the red inducing light and the white test light. Therefore the decay of the contrast effect of the light-adapted retina can not simply be interpreted as a destroying effect by white light.

Rate of decay of contrast effect and adaptation levels

The question arose as to how the rate of decay of contrast effect depended on the adaptation level of the retina.

We supposed that the contrast effect would decline at a greater rate when the adaptation level was raised. But in fact it was found that the rate was

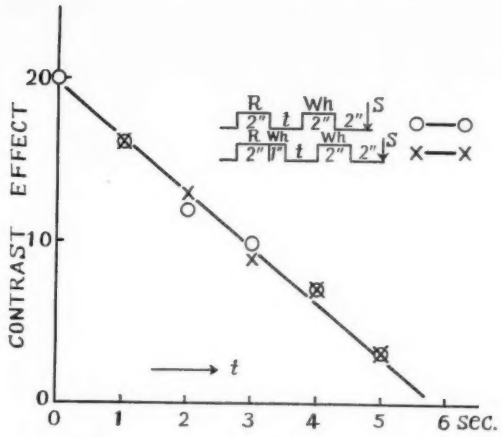

Fig. 4. Experiments showing no effect of interposed white light of 32 lux upon the rate of decay of retinal induction. Curves of decay with and without interposed white light coincide with each other.

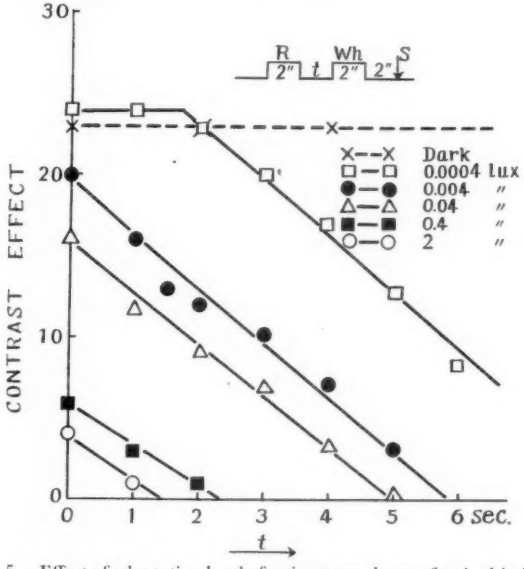

Fig. 5. Effect of adaptation level of retina upon decay of retinal induction. Ordinates: Contrast effects. Abscissae: Time interval between inducing and white test lights.

almost independent of the adaptation level. An example of the results indicating this relationship is shown in Fig. 5.

In this experiment the intensity of adapting light was varied from 0.0004 to 2 lux. The magnitude of contrast effect becomes smaller as the adapting light becomes stronger, but the rate, at which the contrast effect declines, remains almost constant. This is expressed in the fact that the lines of decay are parallel to one another, regardless of the adaptation levels.

At a higher adaptation level the contrast effect disappears earlier. This is not because the rate of decay is greater, but because the initial magnitude of contrast effect is smaller. It is an open question how to interpret this phenomenon.

Discussion

The important fact confirmed in this experiments is that the crest time of the excitability curve is invariant irrespective of the adaptation state of the eye.

The crest time was found 1, 2 and 3 sec. for red, green, and blue lights respectively in the eye adapted to white light of various intensities. In the light-adapted eye, the magnitude of ζ is smaller than in the dark-adapted one.

The contrast effect is decreased by light adaptation. These changes are significant effects caused by light adaptation.

Reduction of photosensitivity has been regarded as a synonym of light adaptation. The present investigation has shown that the magnitude of both ζ and contrast effect is reduced by light adaptation in addition to reduction in photosensitivity. As has been shown above, retinal induction as expressed by contrast effect disappears more rapidly in the light-adapted retina.

This fact seems to be important for the retina to respond to a new stimulus, and in line with the fact that CFF is higher in the light-adapted retina than in the dark-adapted[4]. It is certainly a curious finding that the decay time of the contrast effect was not affected by the strong white light interposed between the inducing and the test lights, while it depends greatly upon the adaptation level of the retina. As has been stated above, the same was true of the dark-adapted retina (Nakagawa & Kohata[5]). These findings indicate that the effect of a temporary white-light stimulus and that of long-lasting adapting light are not the same.

Summary

Retinal induction of the light-adapted human retina was investigated.

1. When red, green and blue lights are used for preillumination in the light-adapted state of the retina, the peak of the curve of electrical excitability lies at 1, 2 and 3 seconds respectively just as in the dark-adapted retina.

As the intensity of the adapting light increases from 0.004 lux to 30 lux, the magnitude of the curve decreases steadily.

2. The curves of retinal induction are not qualitatively different from those obtained from the dark-adapted eye.

The retinal induction decays with time in about 5–6 seconds in the retina adapted to a weak general illumination.

This is in contrast with the fact that the retinal induction of the dark-adapted retina remains unchanged for about 20 seconds.

3. The magnitude of retinal induction or contrast effect is smaller as the adaptation level of the retina is higher.

4. In the retina light-adapted to a general illumination of 0.004 lux, the white light of 32 lux interposed between the inducing light and the white test light does not affect the direct induction.

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From Color Induction to Brightness Induction

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Introduction

Using the method of electro-stimulation, Motokawa[1)-3)] elucidated physiological laws of retinal induction. The retinal induction is classified into two groups, that is, direct induction and indirect induction. The former corresponds to successive contrast and the latter to simultaneous contrast.

Retinal induction was measured in the dark-adapted retina by Motokawa and Katayama *et al.*[6)] and little information is available about the same phenomenon in light-adapted eye.

The present experiment was undertaken to elucidate the properties of retinal induction in the light-adapted retina.

Experimental

Method

The method has been described in detail in the previous papers cited above, so that only the principle of the method will be mentioned here. All the experiments were carried out after 10 minutes light adaptation.

The electrical supernormality of the eye after short exposure to colored light was measured taking the least perceptible phosphene as an index. The effect of light is expressed by ζ, which is defined by the formula:

$$\zeta = 100\ (E - E_o)/E_o$$

where E and E_0 represent electrical excitability (reciprocal of threshold) determined with and without preillumination respectively.

As was shown by Hamada[7)], ζ-time curves for colored lights are qualitatively the same under light adaptation as under dark adaptation.

The curve has a maximum at 1, 1.5, 2 and 3 seconds respectively after the end of illumination, when red, yellow, green and blue lights are used for preillumination.

The direct induction caused by a colored stimulus is measured by a contrast effect which is defined as the difference between the maximal ζ value

obtained by the sequence of stimuli, colored and white and the corresponding ζ value obtained by the same white light alone.

To control the degree of light adaptation, the illumination on a white screen standing vertically in front of the subject was varied from 0.006 lux to 30 lux. The distance between the screen and the eyes was always 40 cm. In the middle of the screen, there was a small opening subtending 2° in visual angle which was illuminated from behind by colored or white test light. Spectral lights from an equal energy spectrum were used, and their intensity was controlled by means of neutral filters whose transmission factors were 0.7, 0.7^2, 0.7^3, etc.

Results

According to Hamada's[7] experiment the "crest time", i.e. the time from the end of illumination to the crest of the ζ-time curve was 1, 2 and 3 seconds for red, green and blue lights respectively, and this relation was not changed by light adaptation.

The direct induction set up in the light-adapted eye was found qualitatively the same as in the dark-adapted eye; the ζ-time curve obtained by the sequence of colored and white lights has the same character as the curve obtained by isolated presentation of the complementary color. For instance, the curve for the white test light preceded by yellow light is qualitatively the same as the curve for blue light alone.

The height of the curve was found greatest when the colored light was followed by the white test light without any interval between them. The magnitude decreased rapidly as the interval was lengthened. In view of these findings by Hamada, colored and white lights were presented without interval in the following experiments.

Influence of the intensity of adapting light upon the direct induction

Blue light of 490 mμ was used as inducing light the intensity of which was kept constant throughout the experiment. The data are illustrated in Fig. 1 in which contrast effects are plotted as ordinates against the logarithm of the intensity of adapting white light as abscissas.

The inducing colored light and the white test light were always 2 seconds in duration and the latter was 100 lux in intensity. The adapting light was varied from 30 lux to 0.006 lux. As can be seen in the curve connecting empty circles, the contrast effect increases as the level of light adaptation is raised, and reaches a maximum at an adaptation level of 1 lux. Further elevation of the level causes a strong reduction of contrast effect, and no induction can be shown in the too strongly light-adapted eyes. The curve connecting solid circles refers to a similar result obtained when the interval between both stimuli was 0.5 sec.

Influence of the intensity of inducing colored light upon the contrast effects

Next the influence of the intensity of inducing light upon the contrast effect was investigated. The experiments were carried out in the adaptation state most favorable to retinal induction, that is, the intensity of adapting light was confined to 1 lux and the inducing light and the white test light were presented

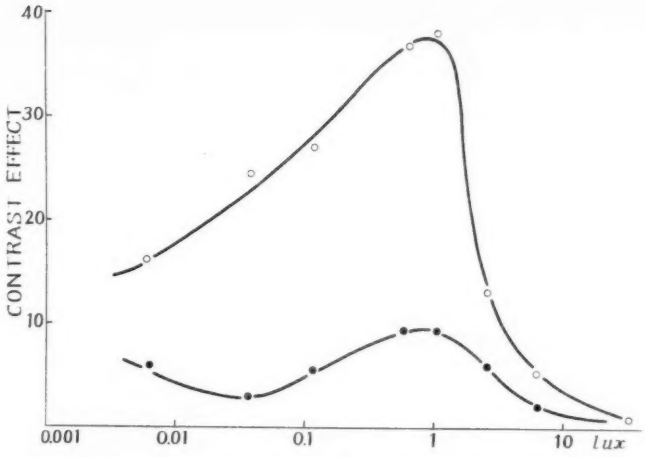

Fig. 1. Dependence of direct induction upon intensity of white adapting light. Intensity of inducing light was fixed. Ordinates: Contrast effects. Abscissas: Logarithm of intensity of adapting light. Curve connecting empty circles refers to experiment carried out without interval between inducing and white test lights. An interval of 0.5 was allowed between both stimuli for curve connecting solid circles.

in succession without interval.

Figs. 2, 3, 4 and 5 were obtained with colored lights of 470, 530, 585 and 630 mμ respectively. The curves drawn by thin continuous lines represent ζ-time curves obtained by the sequence of colored and white light stimuli, the one connecting empty circles refers to the curve obtained by the white test light alone. The number on each curve represents the exponent of the transmission factor of the filter used. For example, it was 6 when the factor was 0.7^6. Therefore the greater the number, the lower the intensity.

The curves labeled 0, 1, 2, 3 and 4 have a maximum at 1.5 seconds. A similar curve having a maximum at 1.5 seconds would be obtained by a pre-illumination with yellow light alone.

The height of curves decreases as the intensity of the inducing blue light is decreased. When the intensity was reduced further, the character of the curve changed entirely as is shown by the broken curves labeled 5 and 6 in Fig. 2. When the intensity was reduced further, the form and size of the curve changed greatly again, as is shown by the heavy curve marked by crosses in the same figure.

Corresponding to the first change the subject informed that the inducing patch lost its coloration; it appeared as grey. The second change occurred when the intensity of the inducing light was so much reduced that it caused almost no light sensation, because the curve obtained coincides with the one

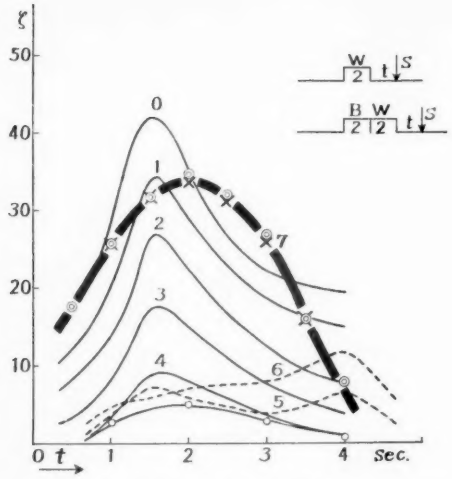

Fig. 2. Curves of direct induction by blue lights of various intensities. Ordinates : ζ values. Abscissas : Time after the end of illumination of white test stimulus. Full explanation in test.

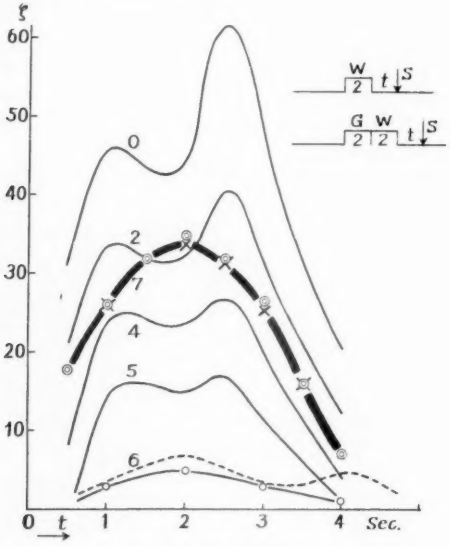

Fig. 3. Curves of direct induction by green lights.

obtained by the sequence, black and white stimuli. (cf. curve marked by crosses

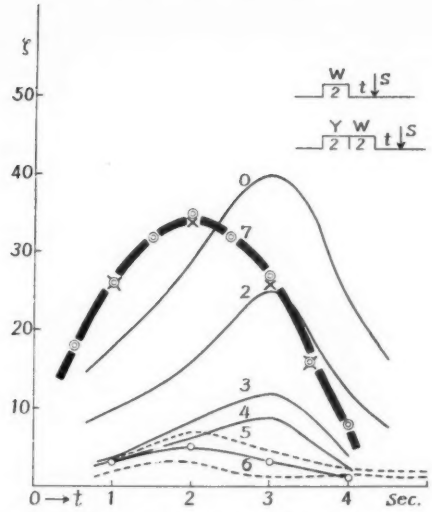

Fig. 4. Curves of direct induction by yellow lights.

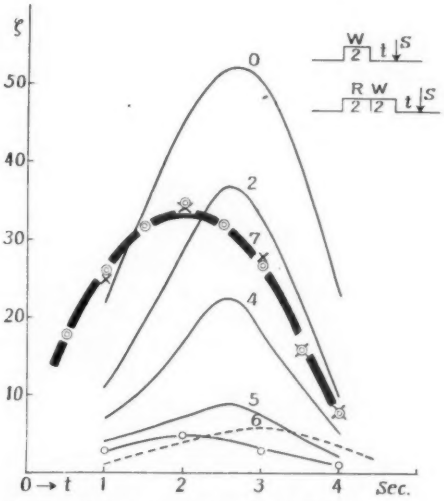

Fig. 5. Curves of direct induction by red lights.

and by double circles). These curves represent the effect of black-white contrast or brightness contrast.

As can be seen in Fig. 3, the same relation was corroborated further by green inducing light. In this case, the intensity of adapting light was confined

to 1 lux, too. The ζ-time curves have two peaks at about 1 and 2.5 sec. as if they had been obtained by purplish light complementary to the green light.

When the intensity of inducing light was below a certain limit, an atypical curve such as indicated by broken line, was obtained. No chromatic effect could be perceived at this intensity. When the intensity was decreased further the curve connecting crosses was obtained in good agreement with the curve marked by double circles for the successive stimuli, black and white.

In Fig. 4, yellow spectral light was used as inducing light. In this case the curves have a peak at 3 seconds, like a curve for isolated presentation of a blue patch.

In Fig. 5, red light was used as inducing light. The curves have a crest at about 2.5 seconds, as if blue-green light alone was used.

In all these experiments two kinds of critical levels of intensity have been perceived, at which a remarkable change occurred in form and size of the ζ-time curve. The first change which can be seen in the broken curves consists in a shift of the maximum, and no remarkable change in the height of the curve is seen yet at this stage.

The second change is more remarkable than the first because the magnitude of the curve grows abruptly. In this sense some discontinuity can be seen between the first and the second changes. The gradation of intensity was made in equal logarithmic steps in the above-mentioned experiments. The second change occurred between the intensities 0.7^6 and 0.7^7.

In the following experiments the intensity was graded in smaller steps between 0.7^6 and 0.7^7 in order to see the degree of discontinuity concerning the change in question. The results are shown in Fig. 6, in which ζ value at 2 seconds are plotted against the intensity. As can be seen in this figure, an abrupt change occurs in ζ values between certain narrow limits of intensity.

The range of intensity at which the discontinuous change occurs, seems to be different from color to color.

For colored light to become equivalent to black it was necessary to reduce the yellow light (Y) most strongly and the blue light (B) the least, red (R) and green (G) being intermediate between them. This order is the same as the order of brightness of the colored lights used. It seems therefore, possible to study the brightness of colored light in this way without resort to any brightness sensation.

Discussion

According to Motokawa, the direct induction in the dark-adapted human retina remains constant for about 20 seconds after removal of inducing light. Hamada[7] showed that the direct induction in light adaptation decreases rapidly and disappears in several seconds. By the latter fact it is suggested that light adaptation facilitates the dissipation of retinal induction. As can be seen in Fig. 1, no retinal induction can be proved at intensities higher than 30 lux of adapting light, although no time was

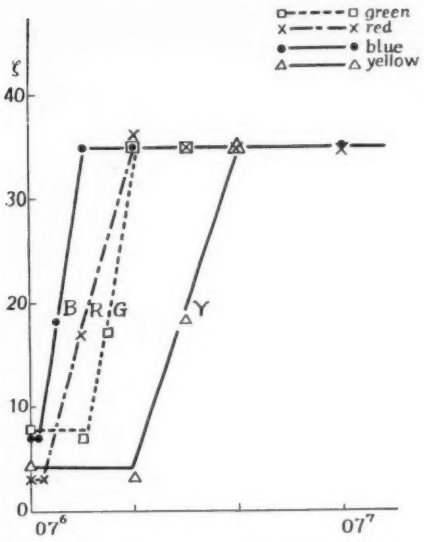

Fig. 6. Abrupt change from color induction to brightness induction. Ordinates: ζ values at 2 seconds. Abscissas: Intensity of inducing colored light. B,R,G and Y stand for inducing spectral lights of 490, 630, 530 and 585 μm respectively.

allowed between the inducing and the white test lights. This may be partly due to rapid dissipation of retinal induction, and also partly due to the circumstance that the intensity of the inducing light was not sufficiently strong against the background illumination. At the given intensity of the inducing light retinal induction increased as the intensity of adapting light increased from 0 to 1 lux (see Fig. 1). In short, it may be concluded that there is an optimal intensity of adapting light for the retinal induction to be caused by inducing light of a given intensity; the optimal intensity of adapting light must be higher with increasing intensities of inducing light.

When the intensity of adapting light was fixed at 1 lux and the intensity of the inducing light alone reduced, retinal induction decreased gradually till it disappeared at a certain intensity below which no sensation of hue was evoked by the inducing light (see Figs. 2–5).

When the inducing light was decreased sufficiently to be perceived as a black stimulus, a curve of quite different character was obtained. This curve represents a curve of brightness induction obtained by a sequence of stimuli, black and white. To obtain this kind of curve the yellow light, the brightest one of all the lights used had to be most strongly reduced in intensity. On the contrary a reduction of the least degree sufficed for the

darkest blue light. It is to be noted that the transition from color induction to brightness induction occurs within a narrow range of intensity.

The character of the curve obtained in the transitory range is somewhat similar to the twin process discovered by Oikawa[4)-5)]. The latter process is seen in the periphery of the retina when colored light appears colorless.

Summary

Retinal induction was studied in the light-adapted human retina by the method of electrostimulation.

1. The properties of retinal induction are qualitatively the same as in the dark-adapted retina except that its dissipation is more rapid.

2. There is an optimal intensity of adapting light for production of retinal induction.

3. Color induction occurs when the intensity of inducing colored light is above the color-threshold.

4. Below the color-threshold a colored patch may appear dark against the background. Then a curve of induction is found quite different from the curve of color induction.

A similar curve is obtained by a sequence of stimuli, black and white (brightness induction).

5. The critical intensity of inducing light changing from color induction to brightness induction is higher for blue and red lights and lower for yellow and green lights.

6. In the transitory range of intensity from color induction to brightness induction a curve of certain type belonging to neither of those stated above is obtained.

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An Accurate-Simplified Haldane Gas Analyzer

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The apparatus depicted here is our version of a simplified Haldane Respiratory Gas Analyzer which preserves the accuracy of the original machine, but is much less expensive, simpler to operate and easier to fabricate. The cost of constructing this particular machine did not exceed $35, of which $20 went to purchase the Haldane burette itself.

The most unique aspect of this machine is the complete elimination of the " thermobarometric compensator " from the circuit, a justification for which will appear in the body of the paper along with a detailed discussion of the analytical procedure. With the apparatus described herein we are able to complete a single analysis in 8 minutes. On good days, and all technicians have such days, we can successfully manage four duplicate determinations per hour.

Our laboratory files indicate replicate analyses of carbon dioxide within .02–.03%. Although the range for oxygen determinations is usually somewhat greater than Haldane claimed for his original machine (.02%), our oxygen determinations seldom vary by as much as .05% and are usually much closer than this. These figures compare quite favorably with the work of other laboratories.

Construction

The standard Haldane burette is usually delivered with a stopcock at the lower end and the tube in this region is usually quite constricted. After calibration, we remove the stopcock and restricted portion of the tube, which does not affect the calibration at all, in order to speed up the flushing action in the burette. The calibration technique suggested by Croton[1] is an excellent method.

The apparatus as a whole is mounted on a piece of half-inch plywood about 75 cm. in height with a baseboard about 25×80 centimeters which gives it stability. The center of the board is cut out (see dotted lines) to permit light

from a 15-watt neon bulb to shine through the burette. To insulate the burette, a piece of opalescent blue or white glass is placed between the light and the burette water jacket. Behind stopcocks (A) and (B), holes of about one inch in diameter are bored to permit the apparatus to lie flat against the board. On the upper right-hand corner a " hook-eye " such as one uses to latch a door must be screwed to the board and a long piece of stout cord strung through it and down the back of the board. Behind the board the string is tied to a 50 m/. polyethylene bottle which acts as a counterbalance for the leveling bulb (L). The bottle can be filled with some surplus mercury. The hook-eye and string should permit some drag on the lip of the board for smooth action during minor adjustments of the leveling bulb. The leveling bulb should be attached to the string with some type of a hook so that it can be easily detached during the time the burette gases are being flushed into the absorbing solutions.

There are many different methods of mounting the flushing arms (B-O) and (B-K), but the simplest method is to drill small holes directly behind the arms themselves and wrap soft aluminum or copper wire around the tubing, which is then twisted together behind the board. This simple set-up permits ready disassembly of the apparatus for cleaning.

The U-tubes K and O, which hold the absorbing solutions in this set-up, are arranged so that they can be moved up and down a centimeter or so during the course of the analysis or clearing operation. The reason for this will be explained in detail later.

The clamps used to hold the flushing chambers were found in a hardware store and are often used to hold round handled household tools up against a wall. A rubber band twisted across the front assures us that the tube cannot slip out of its own accord. The weight of the absorbing solution itself should never move the containers.

The bore of the U-tubes should be about 30–35 mm. and permit free entrance of the flushing tubes K and O. If the tubes are too narrow a " capillarity " situation will be created which may expose the absorbing solution to air or permit oil to get in under the flushing chamber proper.

Stopcock A is connected to the flushing arms with clear latex or plastic tubing for ease of disassembly. The sample tube (S) is connected to the burette through stopcock A with a long, snug piece of latex rubber. It should be necessary to moisten the end of the sample tube with saliva to insert it into its proper connection. Do not connect flushing chamber with stopcock B or, do not connect stopcock B to A filling the flushing chambers!

After the absorbing solutions have been poured into their respective containers they should be covered with a layer of paraffin oil. With marks (m-1) and (m-2) in place, the apparatus is ready for use.

Preparing the Analyzer for Use (Clearing)

At this time no sample tube is mounted and stopcock A should be open to room air. Lowering of the leveling bulb L should now draw room air into the burette, where a sample of about 9 m/. should be taken. Proceed as follows:

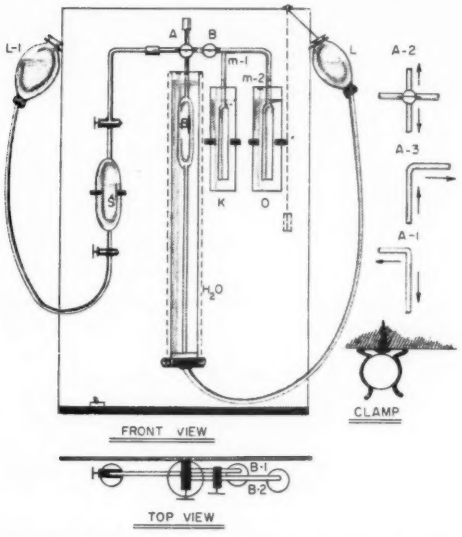

Fig. 1. Ganslen-Sterling modified Haldane analyser in diagram.

Turn stopcock A to position A-3 which closes the burette to outside air and connects the burette with stopcock B. Open stopcock B to the CO_2 absorbing solution in K. Flushing 20 times into K should eliminate any CO_2 in the machine. Return the fluid in K by leveling approximately to the mark m-1 and rotate the stopcock B to position B-2, which will now connect the burette with the oxygen absorber in O. Flush 30 times into the oxygen absorber and level the solution to the mark m-2. Now, switch back to the CO_2 absorber arm K by turning stopcock B to position B-1 again. Flush here four or five times and return again to the oxygen-absorber side. The purpose of this return is to eliminate any oxygen from the arm between B to K which may have been trapped there during the first flushing operation. Now again flush 20 times into the oxygen absorber and level to the mark m-2. If on standing there is no creeping of the mercury in the burette (indicating additional oxygen absorption) the clearing operation has been completed. If there is a noticeable creeping of mercury in the burette between two successive readings, the machine is not clear and an additional 10 flushes into the oxygen absorber should be made.

Balancing the Machine

Assuming that the machine is now filled with nitrogen, we can level and balance the apparatus. Stopcock B should still be in position B-2. Bring the oxygen absorber carefully to the mark m-2, where the fluid level should be steady. Now, turn the stopcock back to connect with the KOH carbon dioxide absorbing side. You may now notice a rise or fall in the level of the fluid around

the mark m-1 in the CO_2 absorbing arm. This rise or fall is due to the presence of a pressure imbalance in the system. That is to say, there is a differential in pressure between the stopcock and O and the stopcock and K. Such pressure imbalances often arise initially because the volume of absorbing solution in the chambers is not exactly the same, or the bore of the tubes on both sides may not be identical. Even the difference in SPG of the two solutions could be at fault.

Let us assume that upon turning stopcock B to K the fluid level fell below the mark m-1. Rather than try to add more solution on this side, which would correct the difficulty, slide the U-tube upward slightly until the fluid level is again at the mark m-1. To test the effectiveness of your correction, now rotate stopcock B back and forth between chambers I and O. If you have carried out the balancing procedure properly there should be no change in the fluid levels in either arm around their respective marks m-1 and m-2. You are now ready to make an analysis.

Analytical Procedures

The machine has now been cleared and switching stopcock B back and forth does not change the level of the fluids in the two absorbing chamber arms around their marks m-1 and m-2. First turn stopcock A toward the sample tube S. Insert the sample tube outlet connections into the rubber sleeve so that it is connected to stopcock A. Hang the leveling bulb for the sample tube. Open stopcock A to position A-1. Upon opening the upper and lower stopcocks of the sampling tube (S), the respiratory sample will now be under positive pressure and free to enter the burette. Since there is some dead space between stopcocks A and the sample tube proper, it is necessary to flush this gas out in order to obtain a representative gas sample. Proceed carefully as follows:

Unhook the leveling bulb L and grasp stopcock A in the left hand. Carefully lower the leveling bulb until the mercury in the burette begins to approach the 6 m*l.* mark, at the base of the broadened area of the burette. Do not lower the bulb rapidly or you may use up too much of your sample in flushing the burette and have none left for analysis. Now, elevate the leveling bulb L and, as soon as the mercury in the burette bulb begins to climb, open stopcock A to room air, position A-2. This procedure will drive out 5 or 6 m*l.* of gas flushed from the arm between the stopcock and the sample tube proper. On elevation of the leveling bulb, the mercury will surge rapidly upward; therefore, as soon as it begins to approach the upper neck of the burette, under stopcock A, turn stopcock A back to the sample and lower the leveling bulb again. *Stopcock A must never be opened to room air unless an over-pressure of gas exists in the burette proper, i.e.*, while the mercury column in rising; otherwise, room air will enter the burette and contaminate the sample. Repeat this flushing procedure four or five times, depending on how much sample you have to work with. On the last flush, attach the leveling bulb to its counterbalance. Stopcock A should still be in position A-1. The next maneuver is critical!

Remove the leveling bulb belonging to the sample bulb from its hook and carefully level the mercury in this bulb with that showing in the sample tube

itself at this moment. To guide this tube it is sometimes desirable to place one finger along the side of the board for friction. Level as accurately as possible, since this step will determine whether you are creating any overpressure or underpressure of the gas in the burette, which gas you are about to analyze. If there is any reason to suspect that you can not level these two tubes perfectly, (and no one can!) apply a slight over-pressure by elevating the mercury in the leveling bulb L-1 a fraction of a cent. Check the mercury level at the base of the burette and, if it has climbed the burette, lower the leveling bulb L to make sure you get at least a 9-m*l*. sample. Turn off the upper stopcock of the sample tube and re-hang this leveling bulb, L-1. There is no need to touch stopcock A at this time.

Since we balanced the machine earlier in clearing we can proceed with the analysis. Turn stopcock A to position A-3, which again connects you with the KOH absorber. You will probably note a slight rise or fall of the KOH solution around the mark m-1 because your sample tube leveling procedure was not perfect. It seldom is. Now, without undue haste, but smoothly, lower or elevate the leveling bulb (L) to bring the solution to the mark m-1 and read your initial gas volume. Flush 20 times, level, and read again as you did in clearing the machine. The carbon dioxide has now been absorbed. Upon turning to the oxygen absorber there should not be any change in the level of the solution around the mark m-2 unless you balanced the machine improperly after clearing it. If a fractional movement of the fluid is noted, don't get excited. Level immediately and read the gas volume. This minor re-adjustment on the oxygen absorbing side, if accomplished rapidly, will not result in any significant loss of oxygen from the sample to the absorber, but any delay here might be disastrous to your results. Flush as described previously in clearing the machine, 50 to 60 total flushes on the oxygen-absorbing side, until no creeping of the mercury in the burette takes place when the leveling bulb has been re-hung. Read your final gas volume. With the completion of this analysis (which takes about 8 minutes for an experienced operator), you will be ready for the next sample without the necessity of the clearing operation. Always check the gas pressure balance in the machine before taking in a new sample and re-balance if necessary. When allowing the machine to stand, leave the burette connected with the oxygen absorber as a matter of general practice, since carbon dioxide is easy to clear out of a machine.

Discussion

The thermobarometric compensator was included in the original Haldane analyzer described by Consolazio, Johnson and Marek[2] to take care of the differential pressure changes in the apparatus arising from or accompanying the exothermic reactions which take place during the absorption of carbon dioxide and oxygen. In order to evaluate this temperature factor the water jacket bathing the burette was fitted with an extremely sensitive Leeds Northrup potentiometer sensitive to .01°

temperature changes. The fluctuations resulted from the analysis itself are tabulated below.

	Readings (mv)	Time	Temperature
Start KOH flush	.964	9:21	24.44
Finish "	.964	9:23	
Start O_2 flush	.965	9:23	24.44
Reflush KOH	.966	9:24	
	.966		
	.966		
Return O_2 flush	.967	9:26	24.50
	.968		
Continue O_2 flush	.969	9:27	24.50
	.969		
	.969		
Analysis complete	.969	9:30	24.50
		Net diff.	.06° Centigrade

It should be pointed out that this net difference in temperature fluctuation resulted after 9 minutes of continuous flushing. The temperature difference is insignificant.

Asmussen[3] found that the use of the thermobarometer in the Scholander apparatus is superfluous and we are convinced that the same situation is true with this apparatus. The principal causes of the gas pressure imbalances in this work do not result from the exothermic reactions during the analysis or variations in the atmospheric pressure, but are the consequence of careless balancing the machine at the completion of the clearing operation or improper leveling of the sample tube leveling bulb L-1 mercury when the sample is brought into the burette. Routine errors involved in Haldane Gas analysis work are admirably evaluated in a fine paper by Renburn and McK. Ellison[4] which should be read by all workers in this field.

As in all gas analysis work, a routine analytical procedure must be perfected. There are probably more errors in taking gas samples from bags than there are in actually making the analysis. Any one intending to do gas analysis work must have some elementary knowledge of the principles of gas pressures. A hasty movement in gas analysis work can easily flood the burette with absorbing solution and require an hour of cleaning before one can get back into operation. This machine is unusually easy to clean.

It is preferable to make the absorber arms short to reduce the dead space in the machine to a minimum, capillary tubing is recommended throughout. The horizontal tubes leading to K and O can be as little as 6 or 7 cm. long, while the vertical leading to the inverted funnel might be about another 8 to 10 cm. By making these arms short, the efficiency

of the flushing operation is improved, but there is also greater danger of flooding the burette with a careless movement. It is desirable to keep the vertical chambers K and O as closely parallel to the burette proper as possible. This will reduce eye movements to a minimum.

I prefer the use of sodium hydrosulfite (8 g.) mixed with sodium anthraquinone beta sulfonate (1.5 g.) and a crystal of ferric chloride for absorbing oxygen. The solution is mixed with 50 m*l*. of warm (50°C) 14% KOH solution and cooled under running water before being added to the machine. When this mixture is absorbing oxygen, a distinct white crust forms on its surface and may even coat the tube of the absorber arm, but as the oxygen absorption is completed the crust completely disappears. This crust formation is a rough check on the presence of oxygen in the burette during the flushing operation.

In order to reduce the arm stroke during flushing to a bare minimum, keep the rubber hose connecting the leveling bulb (L) as short as possible. The total amount of mercury contained here is thus reduced and faster flushing is possible.

An operator must always take care to see that he does not take in too much sample from the sample tube. Should this sample be too large, more than 9 m*l*., and the leveling action of the sample bulb be too inaccurate, it will be necessary to lower the leveling bulb (L) again to bring the KOH solution to the mark m-1 for the initial gas volume reading and this may drop the mercury out of the calibrated area of the burette. Technically, the larger sample may be better, but practically it may prove impossible to analyze the larger sample.

Conclusion

This apparatus design completely eliminates the thermobarometric compensator. By making the U-tubes holding the absorbing solutions movable, overpressures and underpressures noted in clearing the machine can be compensated for in this apparatus. The principal cause of the initial internal differential pressures in the machine is the inability of an operator to level the mercury of the sampling bulb, and the leading bulb L-1, in perfectly. This situation sets up a differential pressure situation in the burette which evidences itself upon switching to the carbon dioxide absorbing solution and must be compensated for immediately.

For a detailed discussion of errors in respiratory gas analysis worker the readers are referred to the work of Renburn and McK. Ellison.[4]

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On the Achromatic Zone around the Spot of Mariotte

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Introduction

Mariotte considered that the blind spot discovered by him in 1668 was due to the entrance of the optic nerve. But later, a number of different interpretations were presented against his view. Paquet[1] and Volkmann[2], opposing Mariotte's view, believed that the entrance of retinal vessels alone was blind. But Griffin[2] found that not only the entrance of the optic nerve but also the area immediately surrounding the spot were blind relatively and that the diameter of the blind spot varied according to sorts of the stimuli used. These findings were comfirmed by Weber,[2] Bjerrum,[2] Meisling[2] and Groenouw[3].

Ferree,[4] Berens,[5] Saubermann,[7] Ebbecke[8] and others reported that the limit of the spot depended on various factors such as the size and the brightness of the test object, the color of the background, etc.[9,10] The form of the spot does not always coincide with the form of the papilla as observed with an ophthalmoscope, especially when the spot is determined by a colored test object.

Roslavzev[11] reported that the blind spot became greater in size gradually when the eye was covered with red or green glass. Johanson, Ovio, Polimanti,[2] Haycraft[12] and Van der Hoeve[13] maintained that there was a narrow zone relatively blind to colors surrounding the area blind to white. For example, Haycraft found that this blind zone was narrower for blue, yellow, red and green in the order. According to Van der Hoeve, there is a zone blind to white and beyond this zone the retina is relatively blind to color.

Haycraft determined the extent of the blind zone to colored stimuli around the spot, using a colored patch on the black ground in daylight illumination, and found the color-blind zone to be fairly concentric with the spot. According to Haycraft, a red test object first appears as gray just at the margin of the blind spot, and when moved further from the edge

of the spot, it is seen as yellow, then as yellowish-orange and finally red, green first appears gray, then bluish, yellowish and finally green.

This relation is, as pointed out by Stewart[14] and by others,[15),16)] similar to the color sensation in peripheral regions of the retina.

The most investigations mentioned above were done either under daylight adaptation or in dimly illuminated rooms. No information is, however, available about the color-blind zone of the dark-adapted eye. So we have undertaken the present investigation on the color-blind zone of the dark-adapted retina.

Experimental

Method

The colored lights used were red (650 mμ), green (530 mμ), yellow (580 mμ) and blue (470 mμ) lights obtained from an equal-energy spectrum. The intensity of the spectrum was such that the red light of 650 mμ was 350 times as strong as the threshold intensity of the dark-adapted fovea.

A test patch of definite size was presented on a vertical screen standing at a distance of 100 cm. in front of the subject's eye. The left eye was used, and the other was covered. All the measurements were carried out in a dark room after preliminary dark adaptation of about 30 minutes.

The blind spot was mapped, as usual, by means of a campimeter under weak general illumination of 1.5 lux and then detailed measurements on the extent of colorless zone and others were carried out as follows: As the test patch was fixed at a certain point on the vertical screen, the fixation point, a small red light, was moved from the fovea towards the nasal periphery, till the test patch became invisible. In this way the blind spot was mapped.

After the blind spot had been determined, the color-blind zone around it was examined in the dark after 5 minutes of light adaptation to 6.4 lux.

Taking a point at a distance of 15°30′ in visual angle from the fovea on horizontal meridian as the origin, the limits of the color-blind zone were searched in four directions. Departing from the edge of the blind spot, the patch which had been colorless began to be tinged with the proper color, say reddish with the red patch and greenish with the green patch, finally the proper color appeared at a certain point. This point was regarded as the margin of the color-blind zone.

The extent of the color-blind zone depends on the size of the test patch. So measurements were carried out with the test patches of various sizes ranging from 5′ to 100′ in visual angle. And the extent of this zone also depends on the intensity of stimuli and the state of adaptation, so the intensity of the test stimulus was also varied by means of a neutral wedge, but the duration of exposure was fixed at one second throughout the present experiment, the interval between successive tests being 6 seconds. To use a longer exposure time is undesirable because the sensitivity to color rapidly decreases (adaptation).

Results

1) *The effect of the size of the test patch on the color-blind zone*

Typical examples of the color-blind zone determined after 30 minutes dark adaptation are shown in Fig. 1. The size of the test patch used was 60′ for red light (Fig. 1A) and for green light (B), but 20′ for yellow light (C) and for blue (D).

As will be mentioned later, no appreciable color-blind zone could be obtained with a yellow patch of 60′ or a blue patch of the same size.

As has been stated above, the extent of the color-blind zone depends on the size of the test patch. This relation is illustrated in Fig. 2 in which the sizes of patch were 10′, 20′, 40′, 60′ and 100′ for red and green lights but 5′,

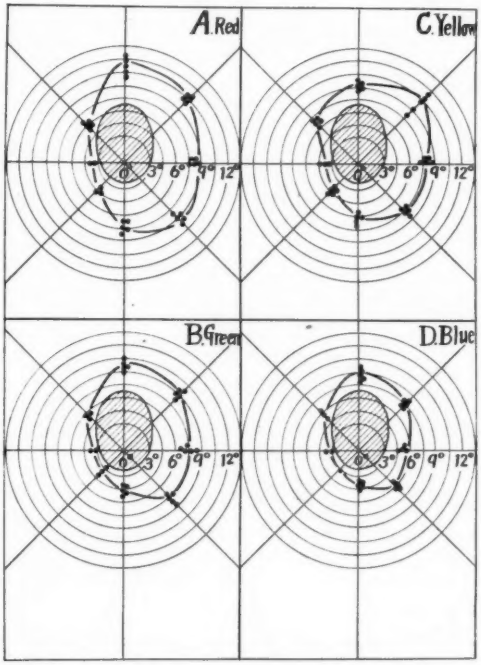

Fig. 1. Typical color-blind zone around the spot of Mariotte (shadowed). A and B were tested by red and green patches of 60′ in visual angle. C and D by yellow and blue patches of 20′ in visual angle.

Outer limit of zone is represented by continuous curve connecting points measured. Each circle represents distance in visual angle from center of blind spot.

10′, 20′ and 40′ for yellow and blue lights. In this figure, the visual field of each color is shaded. When the test patch is too small, for example 10′–20′ for red

light, the peripheral edge of the red visual field is too far apart from the blind spot, so that no color-blind zone is formed. The proper color field extends pregressively towards the blind spot and further beyonds it, as the size of the test patch is increased, but a colorless zone remains around the blind spot so long as the size is not too great (see Fig. $2A_4$). As the size is increased further, the zone becomes progressively narrower until it disappears (see A_5).

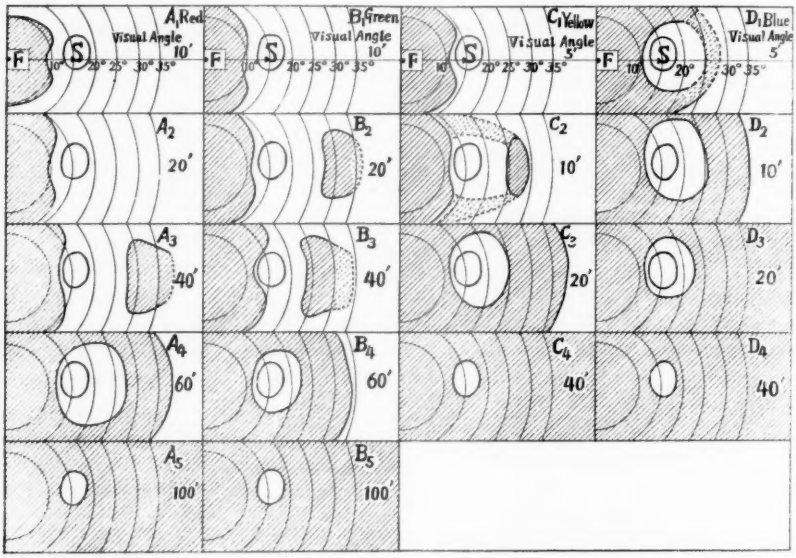

Fig. 2. Visual field for colors determined with test patches of various visual angles. Visual field area is shadowed. S : blind spot, F : Fovea centralis.

Similar changes can be observed for green and yellow lights, but the formation of zone occurs at a much smaller size of test patch for yellow lights (see C_3).

The blue color field is so great that a colorless zone is obtained only with the smallest patch used of 10′ visual angle. (See D_2).

2) *The effect of dark adaptation on the color-blind zone*

After a preliminary light adaptation to a general illumination of 6.4 lux for 5 minutes, the extent of the color-blind zone was measured in the course of adaptation. The measurement was carried out at the 20th, the 40th, the 60th and the 80th minute after onset of dark adaptation.

The results are shown in Fig. 3. To begin with the result by the red patch, there is no blind zone immediately after the onset of dark adaptation, but after 20 minutes a considerably wide color-blind zone can be seen (Fig. 3,A). The extent of the zone increases rapidly till about the 60th minute after the onset of dark adaptation, but no further increase is seen for further dark adaptation.

It is worthy of special mention that at the 80th minute for red and at the

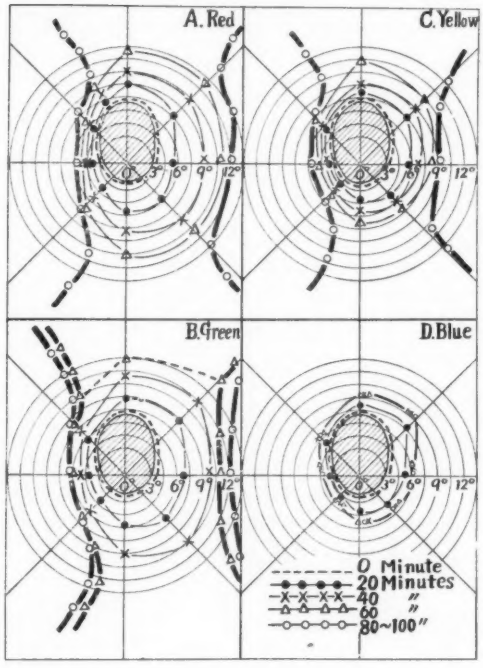

Fig. 3. Color-blind zones measured in various states of dark adaptation. A and B were tested by red and green lights of 60′ respectively, C and D by yellow and blue lights of 20′ in visual angle. The blind zone is mapped at the 20th, 40th, 60th, 80th and 100th minute after onest of dark adaptation.

60th minute for green the color-blind zone becomes open in the vertical meridian.

Such an effect of dark adaptation was only little for blue (Fig. 3,D).

In A and B of Fig. 3, patches of stimuli used are 60′ in visual angle. In C and D they are 20′ in visual angle.

3) *The effect of the intensity of test light on the color-blind zone*

The dependence of the color-blind zone on the intensity of stimulus was further investigated.

The result can be seen in Fig. 4. As the intensity is increased, the zone contracts around the blind spot, and this effect is most remarkable for red and green lights. On the contrary, no significant change can be seen in the zone for blue light.

The intensity of lights were reduced by means of neutral wedges whose transmission factors were 0.7^2, 0.7^5, 0.7^8 and 0.7^{10}. Examinations were carried out with the eye dark-adapted for 30 minutes.

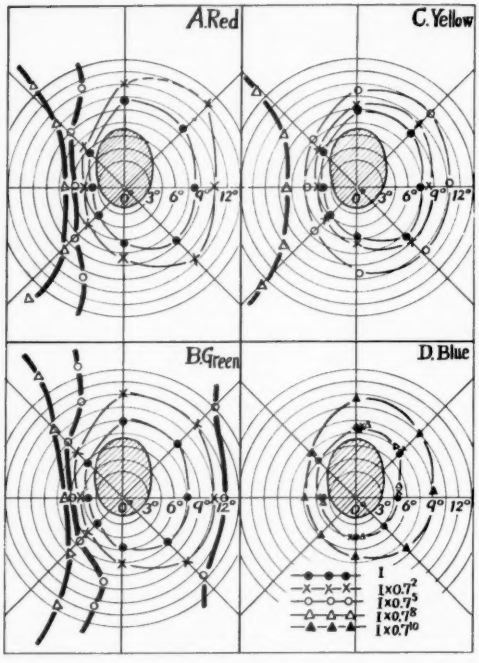

Fig. 4. Effects of intensity of colored light on color-blind zone. Relative intensities used were 0.7^2, 0.7^5, 0.7^8 and 0.7^{10}. Red and green fields were tested by a patch of 60′ : yellow and blue fields by a patch of 20′ in visual angle.

Discussion

The visual field for color is dependent on a number of factors, as has been shown by many authors[17)–19)]. The extent of the color field is especially unstable in peripheral regions of the retina.[20)–22)]

The color sensitivity of the retina around the blind spot is similarly unstable, as has been demonstrated above. Furthermore, Kravkov reported the influence of acoustic[23)–24)] and olfactory stimuli on the color sensation and showed that sensations of green and red were more strongly affected by these stimuli than those of yellow and blue. Roslavzev[11),28)] found the diameter of the blind spot, which was greater for red and green lights, to be varied by the action of noise. These findings suggest the unstable character of red and green sensations around the blind spot.

In the present experiment the weakness of red and green sensitivities has been demonstrated by greater color-blind zone and its greater liability to various effects.

The blind zones for yellow and blue lights are far narrower and more resistent to various influences.

In determination of the margin of the color-blind zone, I used at first the method of displacing the fixation point which was followed by the subjects' eye, but soon I became aware that this method was unsuitable, because the color sensation around the blind spot was extremely sensitive to eye movement. Therefore the fixation point was diplaced step by step, and at each position the color sensitivity was tested by exposing the retinal location in question to the test patch for about 1 second. And an interval of 6 seconds was allowed between successive exposures. The color-blind zone around the spot was mapped in this way.

The procedure for determining the limits of the color blind zone was repeated several times at each point and the average of obtained values was adopted as the value for that point.

It has been mentioned above that the color-blind zone becomes open at the vertical meridian, when the visual angle of test patch is reduced below a certain limit, or when the eye has sufficiently been dark-adapted.

I am not certain whether or not this is due to the retinal vessels which run in the vertical direction in the neighborhood of the optic disc. Perhaps this phenomen is not dependent upon the retinal vessels alone, because this never happens with the color-blind zone for blue light.

Compared with the data obtained by Haycraft, the aspects of the color-blind zones obtained in the present experiment are far more complicated. The difference in the results must be due to the difference of the experimental conditions in both experiments, especially it must be kept in mind that Haycraft's experiment was carried out in a light-adapted state, while my own was performed under dark adaptation.

The color-blind zone for all sorts of color are not concentric with the blind spot, but more extensive towards the periphery than towards the fovea. This may partly be due to the optical asymmetry that the condition of illumination is not the same[25] on the central and the peripheral sides of the blind spot, but the difference in retinal structure must be more significant for this asymmetry in extent of the color-blind zone.

In my investigation it was observed that some older subjects showed an absolute or a relative scotoma in the midperiphery and in the oblique meridian of the spot, although no sign of degeneration of the retina could be perceived ophthalmoscopically.

It is said[26),27)] that the visual field especially the rod-field shows a shrinkage or a contraction with age. The scotomata which can be perceived in the neighborhood of the blind spot by detailed functional examination may be related with the shrinkage of the visual field.

SUMMARY

The color sensitivity to four spectral colors, red, green, yellow and blue around the spot of Mariotte was investigated under dark adaptation of the eye.

1. It was found that there is a color-blind zone around the blind spot, and that the zone is wider in the following order: Red>green>yellow>blue.

2. The zone is not strictly concentric with the blind spot, but wider towards the periphery than towards the fovea.

3. The extent of the zone increases by dark adaptation, especially in the vertical meridian and the upper and the lower parts of the zone rupture at a certain stage of dark adaptation.

This phenomenon is especially remarkable for red and green lights.

4. Reduction of the visual angle of the test patch has an effect similar to dark adaptation on the form and size of the color-blind zone.

5. The zones for red and green lights are more sensitive to changes of intensity of test light than those for yellow and blue liglts.

6. In short, the color sense around the blind spot depends on many factors such as the intensity, the wave-length, the size of the test stimulus, the state of dark adaptation, etc.

At the end of this paper, I must respectfully express my gratitude to Prof. Motokawa for his valuable guidance in this work.

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The Influence of Retinal Adaptation on the Electric Flicker Values

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Introduction

It was reported by Abe[1], by Tsukahara & Abe[2] and by Motokawa & Ebe[3] that the alternating current of 20 c.p.s. might be the optimum frequency for electrical stimulation of the nervous tissue connected with rods.

According to Abe[1], the time course of dark adaptation of the electrical excitability determined with the current of 20 c.p.s. was such that at first the threshold remained low and steady for about 5–7 minutes, and then increased rapidly to attain a maximum in about 17 minutes.

A similar time course of the electrical threshold was obtained also by Iwama[4] using a constant current pulse of about 50 msec.

Recently Mita *et al.*[5] and Seto[6] obtained similar experimental results by using the sinusoidal alternating current of 20 c.p.s.

Barnett[7] interpreted a frequency of about 20 c.p.s. to be the optimum one for electrical stimulation of the cones, while the previous investigators[1-4] considered it as the resonance frequency for the rods.

In the present experiment, I studied the effect of adaptation on the electrical threshold of the eye for the alternating current of 20 c.p.s. with a view to contributing something to the question raised above.

Experimental

Method

The electric flicker value (F.V.) of the eye was measured with an apparatus which was manufactured by Toa Denpa & Co. after Motokawa's suggestion. The detail of the design was explained in Motokawa's paper.[8]

A special use was made of the apparatus in the present experiment, as will be explained below.

As can be seen in Fig. 1, a key, which was missing in the original apparatus, was inserted into the output circuit in order to control the

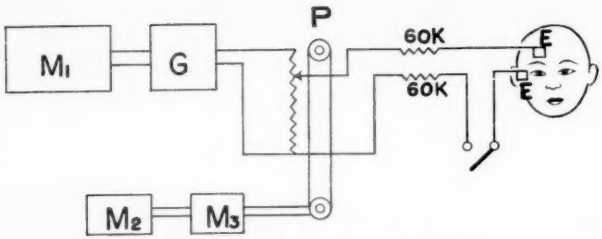

Fig. 1. Apparatus for measuring electric flicker value (F.V.) E : Stimulating electrodes. G : Generator of alternating current of 20 c.p.s. M_1, M_2, M_3 : Synchronous motors. P : Potentiometer whose electric contract is moved up and down by means of M_2 and M_3.

duration of stimulating current. The duration was fixed at 0.5 sec.

The F.V. was determined by the method of trial and error. It was referred to the threshold for disappearance of flicker ; the electrical stimulation which was started from a sufficiently high level was decreased in small steps of 3 μA. until any flicker was no more elicited.

The electrodes were placed on the forehead slightly above the eyebrow and on the homolateral temple. The electrical contact between the electrode and the skin was secured by electrode paste. The other eye was shielded. A patch of ground glass illuminated by tungsten lamps served for light adaptation. A collecting lens and an interference filter were used to produce colored light.

Daily variations of F.V. in my experiment are shown in Table I, which will give an idea of the accuracy in the present experiment.

Results

1) Changes of F.V. in the course of dark adaptation following light adaptation of 10 minutes to 9,800 lux (D-curve)

F.V. was measured in the course of dark adaptation. An example of the results obtained is illustrated in Fig. 2. After 1 min., the F.V. was lowest, and there was no significant rise yet after 3 min. Then it increased slowly till about 8 min. after the onset of darkness, and increased thereafter rapidly to attain a maximum in about 15 min.

This course is almost the same as in the previous papers cited above.

2) F.V.-time curves obtained during continuous illumination with white light of a small retinal area following the same light adaptation as above

In this experiment, a small retinal area at the fovea, 5°, 10° or 20° from the fovea was continuously exposed to white light of 30 lux. The patch used was a circle of 40′ in visual angle.

The curves obtained are illustrated in Fig. 3. The F.V.-time curve

TABLE I

Daily Variations of F.V.

Session		F.V. in μA	
		8′ after onset of darkness	20′ after onset of darkness
1	a.m. 8.00	24	54
	Noon		60
	p.m. 5.00	24	60
2	a.m. 8.00	27	57
	Noon		63
	p.m. 5.00	21	57
3	a.m. 8.00	24	60
	Noon		57
	p.m. 5.00	24	60
4	a.m. 8.00	21	57
	Noon		54
	p.m. 5.00	24	63
5	a.m. 8.00	24	57
	Noon		60
	p.m. 5.00	27	60
Average & standard deviation		24.0±1.89	58.6±2.65

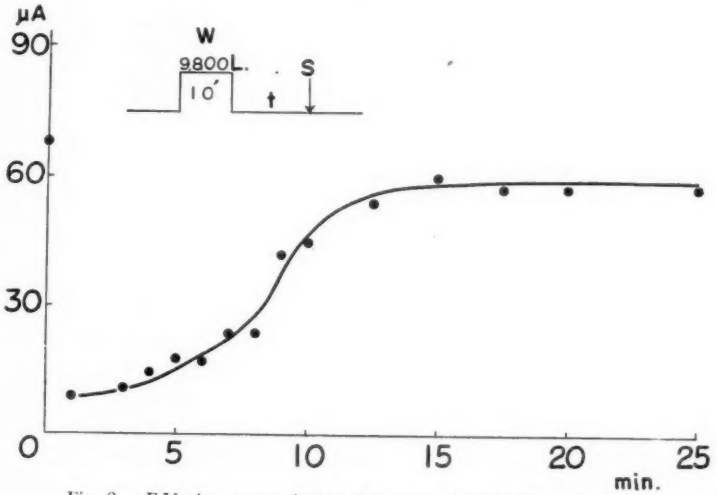

Fig. 2. F.V.-time curve during the course of dark adaptation. Ordinates : F.V. in μA. Abscissae : Time from onset of darkness in min.

obtained by illumination of the fovea ran the same time course as the D-curve obtained above in the complete darkness at its initial stage till 8

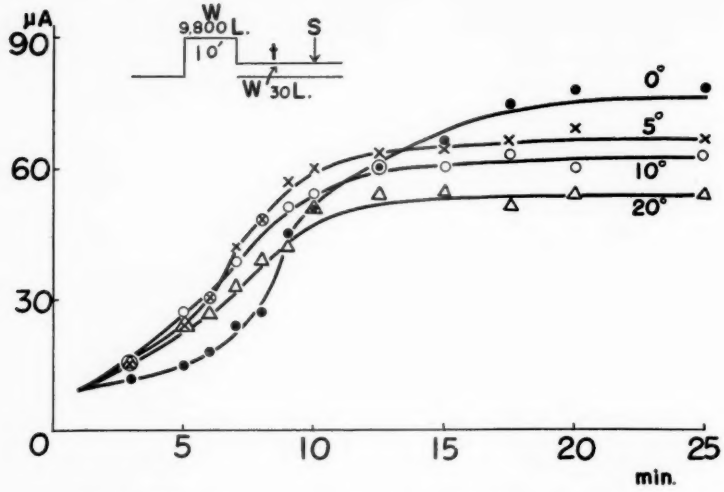

Fig. 3. F.V.-time curves obtained continuous local illumination of a small area of retina with white light of 30 lux following light adaptation. 0°, 5°, 10° and 20° represent eccentricity from the fovea.

minutes, and then rose to a higher level of threshold than the D-curve at the later stage. It is to be noted that the inflection at about 8 minutes is very conspicuous. Such an inflection is, however, only slight or missing in the other curves obtained at the peripheries.

Generally speaking, the curves for the peripheries reach a steady level earlier than the D-curve.

The most characteristic feature is that the farther the location of illumination was moved towards the periphery from the fovea, the lower the steady level of threshold.

3) F.V.-time curves obtained during continuous exposure of a small retinal area to colored light following the same light adaptation as above

A circular patch of 40′ in visual angle was used for continuous illumination, whose illumination was 50 lux without filter.

In Fig. 4, the curves connecting solid circles represent those obtained by illumination of the fovea, and the curves connecting empty circles those obtained by illumination of a peripheral area 40° from the fovea.

As can be seen in Fig. 4 a and b, when blue light (452 mμ) and green light (505 mμ) were used, the two curves referring to the fovea crossed with those obtained by illumination at the periphery about 10 minutes after the onset of dark adaptation. The steady level is much lower at the periphery than at the fovea, the difference is especially conspicuous for

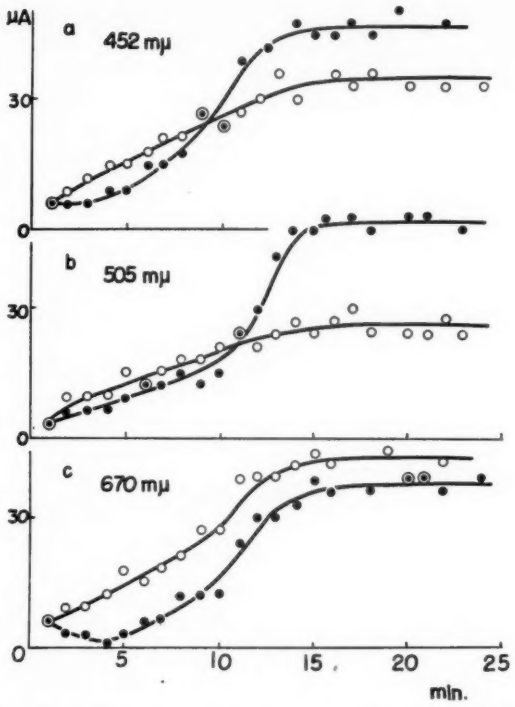

Fig. 4. F.V.-time curves obtained during continuous local illumination of a small area (40′ in visual angle) of retina with colored lights following light adaptation. Curves connecting solid circles refer to illumination at fovea, and curves maked by empty circles to illumination at periphery 40° from fovea.

green light.

The contrary result is seen in Fig. 4 c. When the red light (670 mμ) was used, the curve obtained at the fovea was found to run below the curve obtained at the periphery, and the steady level for the foveal curve was also lower in contrast with the other cases.

4) F.V.-time curves obtained during continuous illumination with white light of a great retinal area following the same light adaptation as above

In this experiment, following the preliminary light adaptation to 9,800 lux the intensity of adapting light was adjusted to 1,000 lux, 100 lux, 10 lux, 1 lux or 0.1 lux, and F.V.-time curves were obtained for each secondary exposure (Fig. 5). For comparison the D-curve is represented by a broken curve.

As can be seen in this figure, there was almost no elevation of threshold

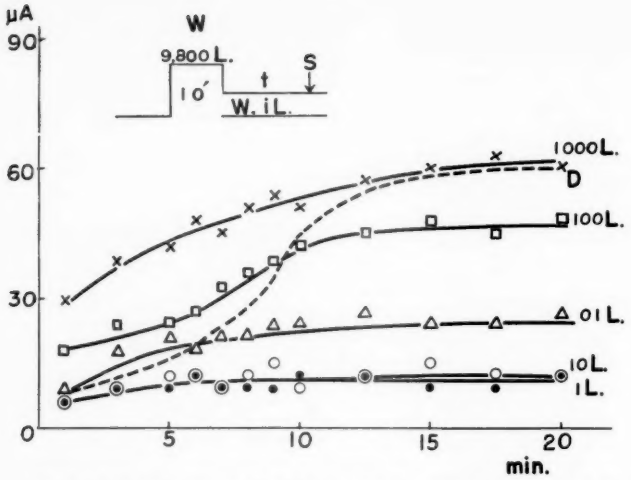

Fig. 5. F.V.-time curves obtained during continuous illumination of a great area (120° in visual angle) of retina with white light of various intensities following light adaptation.

in the two curves obtained by exposure to 1 lux and 10 lux. These two curves were lower than the D-curve. There was a slight increase in the curve obtained by illumination with 0.1 lux. As for the curve for 100 lux the threshold at the initial stage was higher than the D-curve, but the final level was lower than it.

Discussion

Abe[1] determined the F.V.-time curve of the human eye under experimental conditions similar to the present experiment, except for the intensity of adapting light; it was 24,000 lux, that is, higher than in the present experiment. He states that at first the threshold remained low and steady for about 5–7 minutes, and then increased rapidly to attain a maximum in about 17 minutes, but made no graphical representation. Abe's result[1] agrees well with my D-curve; a small difference in time required to reach the final steady value depends probably on the difference of the pre-illuminating intensity. The time to reach the final steady value was found to be about 10 minutes, in Seto's experiment[7], in which the intensities of adapting light were only 1,000 and 5,000 lux. There is no essential difference between the D-curve and those obtained by Iwama[4], Mita[5], Seto[6], although experimental conditions were not identical.

The rapid increase of threshold as is seen at the middle stage of those curves was ascribed by Abe[1] to the dark adaptation of the rods. This

interpretation is supported by the fact that under continuous illumination confined to the fovea, the inflection at the middle stage is especially conspicuous (see the curve 0° in Fig. 3). It is because the dark adaptation of the retinal periphery is not disturbed by foveal illumination. In such interpretation it is assumed that excitation of visual cells, especially rods and in consequence increased activity of the nervous system concerned will lead to a lowering of electrical threshold as measured by the present method. The inflection of the curve disappears when the dark adaptation of the rod is disturbed by peripheral illumination of the retina (see curve 5°, 10° and 20° in Fig. 3). The fact that the final threshold becomes lower as a farther periphery is illuminated, can well be understood from this point of view. No inflection can be seen in the curves for the light of optimum wave-lengths to stimulate the rods, and the final thresholds are very low (see Fig. 4 a and b). On the contrary a very conspicuous inflection can be seen in the curve for red light which does not excite the rods (see Fig. 4 c).

Moreover, the F.V.-time curve obtained during the secondary illumination with 10 lux–0.1 lux which may be regarded as exciting the rods optimally, showed no inflection and their final thresholds were very low (see Fig. 5).

The values of the final thresholds of those curves illustrated in Fig. 5 are very near the value of thresholds in strength-log illumination curves reported by Takahashi[9].

About the earlier stage of the F.V.-time curve, it may be supposed that the responses of the cones and the elements connected with them are responsible for electrical thresholds, because the threshold at this stage was found very low when the fovea was illuminated by the red light.

Summary

The electrical threshold of the eye was measured by the alternating current of 20 c.p.s. with flicker sensation caused by it as an index under various adaptation states.

1. The threshold-time curve during complete dark adaptation following light adaptation of 10 minutes to illumination of 9,800 lux shows the following course: At first the threshold remained low for about 8 minutes, and then increased rapidly to attain a constant level in about 15 minutes. Thus, the curve showed an inflection at about 8 minutes.

2. The curve suffered from a modification when the retina was constantly exposed to a small point of light of 40′ in visual angle following the same light adaptation as above.

The inflection at the middle stage became more obscure and the final level lower as local illumination was moved farther towards the periphery

of the retina.

3. The effect of similar local illumination was investigated with colored lights.

When blue, green and red lights were used for local illumination, it was found that the red light showed an effect similar to white light confined to the fovea, but that the green and blue light acted as white light illuminating the periphery of the retina.

4. Next the effect of exposure to broad illumination was investigated upon the curve following the same light adaptation as above.

The curves by illumination of 0.1 lux, 1 lux and 10 lux were low and almost steady, but those by 100 and 1,000 lux were generally much higher.

From these experiments it is likely that the threshold as determined by the A.C. of 20 c.p.s. is lowered when visual cells, especially rods are excited to some extent.

I am greatly indebted to Prof. Motokawa for his many valuable discussions and kind guidance.

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Studies on Leucocytosis and Leucopenia in Infectious Diseases

Report I

The Hematopoiesis of the Bone Marrow in Leucocytosis

By

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Concerning the genetic mechanism of leucocytosis and leucopenia Naegeli's[1] opinion has been widely adopted. He explained leucocytosis as an expression of sufficiency and leucopenia as that of insufficiency of the bone marrow. Saito experienced during 1940–1941 several cases of typhoid fever with remarkable leucopenia, in which perforative peritonitis had been complicated and the leucocytes increased with lethal prognosis. Hereupon the authors began to harbour doubt about Naegeli's opinion.

In 1942 in 8 cases of relapsing fever Saito found that the impulse of the pathogenetic microbes reached the bone marrow causing fever, the bone marrow became irritated and reacted against them and among the nucleated cells in the bone marrow primitive cells were most irritable and reactive, showing a tendency to fall readily into degeneration or destruction.

In the bone marrow an increase of the cells was seen first in the primitive cells and later gradually in more mature ones. After that, we[2] observed 38 scarlet fever cases in order to research into the genetic cause of leucocytosis. In these cases 46 bone marrow punctures were made.

Experimental

Methods

The bone marrow specimens were taken from the breast bone in the second intercostal space, 3–5 mm lateral from the median line. 0.1 cc each of bone marrow was taken out of it in order to count the nucleated cells and to examine the bone marrow picture in smear preparations which were fixed by May-Giemsa double staining. The blood specimens were taken every day and the bone marrow specimens in 5 cases out of 38 cases regularly once a week by serial

TABLE I

Bone Marrow Picture on Every Day of Scarlet Fever Cases

Day of the illness			2	4	5	6	7	8	9	10	11	12	13	14	20	21
Number of cases			1	7	6	9	5	3	2	2	4	2	1	1	2	1
R.B.G. (Mill.)			3.80	4.425	3.597	3.549	3.488	3.485	3.317	3.377	3.456	3.57	3.25	3.82	3.55	3.25
Hgb. (%)			70	95	78	75.6	78.2	80.3	74.5	71.5	78.7	74.5	76	70	80.5	72
Color-index			0.9	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.2	0.9	1.1	1.1
Nucleated cell count (Thousand)			345.6	491	124.9	153.0	184.8	233.7	270.5	104.8	114.9	249.9	126.0	95.8	59.0	198.4
Leuco- and lymphopoiesis (%)	Myeloblasts		1.8	1.2	1.5	1.2	1.2	1.1	1.5	1.5	1.2	1.1	1.3	1.4	1.6	1.4
	Neutrophils	Promyeloc.	6.5	5.6	5.2	4.8	3.0	3.7	3.8	3.3	3.5	2.4	2.6	2.3	2.9	3.2
		Myeloc.	30.2	16.8	18.1	23.5	19.2	20	20	17.5	14.8	12.2	12.8	16.4	11.4	16.3
		Metamyeloc.	18.6	23.4	22.8	24.9	28.9	30.9	28	28.2	26.7	20.8	17.8	23.6	19.5	20.7
		Rod-shaped	11.3	20.7	17.2	16.7	20.8	21.7	22.1	21	24.4	24.2	24.2	24.5	28.9	23
		Segmented	3.8	6.4	4.3	3.8	4.4	5.4	5.6	4.9	6.6	12.9	7.4	6.2	8.4	12.5
		Total	70.4	72.8	67.6	73.8	76.3	81.6	78.6	74.9	76	72.4	64.8	73.0	70.9	75.7
	Eosinophils		4.30	5.02	6.41	6.45	4.42	3.46	3.00	5.15	5.17	3.95	7.20	3.40	4.50	5.20
	Basophils		0	0.08	0.03	0.04	0.02	0.03	0	0.05	0.07	0.15	0.10	0	0	0.10
	Monoc.		0.80	0.88	1.00	0.84	0.40	0.80	1.65	1.3	0.9	1.45	0.90	1.00	2.35	0.9
	Lymphoc.		19.2	16.8	17.8	13.4	12.6	7.9	11.3	10.5	13.1	17	21.4	13.6	15.4	12.7
Reticulo-endothel (%)	Lymphoid cells		2.3	1.8	2.8	1.5	1.5	1.4	2.1	3.7	2.4	2.3	3.4	4.8	2.3	2.4
	Plasma cells		1.20	1.48	2.88	2.46	3.82	3.5	1.65	3.05	0.97	1.2	0.9	2.8	3.2	1.5
	Macrophages		0	0.04	0.06	0.04	0.06	0.03	0	0	0.02	0	0	0	0	0.1
Erythro-poiesis (%)	Erythroblasts	Basophil	1.0	1.9	2.2	2.52	1.4	2.5	2.4	3.0	2.2	1.9	1.7	2.0	1.1	2.2
		Polychromatic	7.8	8.3	6.9	9.8	6.5	7.2	7.3	10.9	11.9	4.4	10.7	10.7	7.4	9.5
		Oxyphil	1.2	1.4	1.5	1.6	1.2	1.4	1.2	1.3	1.5	0.9	1.0	1.8	0.6	0.8
Megakaryoc.			0	0.3	0	0.22	0	0.33	0	0	0.5	0	0	0	0	0

punctures following recovery. The nucleated cells in the bone marrow were differentiated by Rohr's method[3] since that seems to be the most reasonable in comparison with the method of using peripheral blood specimen.

The nucleated cells appearing in the field of vision during counting 1,000 cells of the leucopoietic system in the bone marrow preparations were differentiated into three groups: reticuloendothelial, erythropoietic and leucopoietic systems. In the blood preparations 500 leucocytes were differentiated.

Results

The nucleated cell counts in the bone marrow (Table I)

The nucleated cell counts in the bone marrow were at a maximum of 345,600 on the second day and at a minimum of 49,100 on the fourteenth day and on the average in the first week 151,700, in the second 171,500, in the third 154,700, showing greater counts in children than in adults in every stage of the illness.

The erythropoietic system (Table I, II, III)

Both in the bone marrow and the peripheral blood the cell counts of the erythropoietic system decreased in the course of the illness. They were greater in adults than in children. The red cell count in the blood was greater than that in the bone marrow. Hemoglobin contents were also greater in the former than in the latter, and these were greater in adults than in children. Color-index decreased in the course of the illness in the peripheral blood, while in the bone marrow it increased slightly in the course, as Table III shows.

In the bone marrow proerythroblasts were 1.6% in the first week, 2.2% in the second and after the third week decreased in percentage. Basophil erythroblasts remained in 1.0–3.0% and did not show a remarkable change. Polychromatic and oxyphil erythroblasts decreased slightly in the course of the illness in the range of 6.0–15.0%.

In our cases erythroblasts never appeared in the peripheral blood through the entire course of the illness.

The leucopoietic system (Table I, II, III)

The white blood cell counts in the pripheral blood ran parallel to the nucleated cell counts in the bone marrow. As the Table III shows, the leucocytes in the blood were in the first week 15,000, in the second 14,000, in the third 12,600, so they decreased in the course of the illness.

The leucocytes in the peripheral blood and the nucleated cells in the bone marrow were more numerous in children than in adults.

In the bone marrow picture myeloblasts appeared at a maximum of 1.8% on the second day and after that they remained in about 1.0–1.6%.

Neutrophil cells. Unmatured myelocytes increased strikingly at the beginning of the illness, and counted 6.5% on the second day, decreasing in the later course of the illness. They counted in the first week 4.9%, in the second 3.3%, in the third 3.0%, and increased more strikingly in children than in adults.

Myelocytes showed a relative increase to 30.2% on the second day, but a rapid decrease to 16.8% on the fourth day. They increased gradually thereafter and showed a maximal value of 23.5% on the sixth day, decreasing in the later

TABLE II

Bone Marrow Picture in Every Week of the Scarlet Fever Cases

Week of the illness			I	II	III	I Child	I Adult	II Child	II Adult	III Child
No. of cases			28	15	3	21	7	12	3	3
R. B. G. (Mill.)			3.636	3.455	3.450	3.493	4.061	3.345	3.926	3.45
Hgb· (%)			77.7	76.2	77.6	74.5	87.4	74.4	83.3	77.6
Color-index			1.1	1.1	1.1	1.0	1.1	1.1	1.1	1.1
Nucleated cell counts (Thousand)			151.5	171.5	154.7	168.7	99.8	184.1	141.2	154.7
Leuko- and lymphopoiesis (%)	Myeloblasts		1.3	1.2	1.5	1.3	1.1	1.3	0.86	1.5
	Neutrophils	Promyeloc.	4.9	3.3	3.0	4.9	4.7	3.4	2.7	3.0
		Myeloc.	20.1	16.5	13.0	20.6	19.0	16.3	17.0	13.0
		Metamyeloc.	24.9	26.3	19.6	25.4	23.7	27.6	21.0	19.6
		Rod-shaped	18.3	23.0	26.9	17.3	21.6	23.2	22.2	26.9
		Segmented	4.6	6.8	7.7	3.6	6.2	5.8	11.1	9.7
		Total	72.9	75.9	70.2	71.7	75.1	76.4	74.2	72.2
	Eosinophils		5.65	4.39	4.73	5.75	5.34	4.63	3.43	4.73
	Basophils		0.04	0.06	0.03	0.05	0.01	0.05	0.1	0.03
	Monoc.		0.80	1.11	1.86	0.77	0.90	1.01	1.50	1.86
	Lymphoc.		15.2	12.7	14.5	16.3	12.2	11.8	17.6	14.5
Reticulo-endothel (%)	Lymphoid cells		1.9	2.3	2.3	1.7	2.0	2.3	2.4	2.3
	Phasma cells		2.50	1.94	2.63	2.19	3.45	1.30	3.50	2.63
	Macrophages		0.03	0.01	0.03	0.03	0.04	0.02	0.10	0.03
Erythro-poiesis (%)	Erythro-blasts	Basophil	2.0	2.2	2.5	2.0	2.2	2.2	2.1	2.5
		Polychromatic	8.0	9.1	8.1	7.5	9.2	9.7	6.6	8.1
		Oxyphil	1.4	1.3	0.7	1.5	1.3	1.3	1.3	0.7
Megakaryoc.			0.1	0.2	0	0.2	0	0.3	0	

course of the illness. They averaged in the first week 20.1%, in the second 16.5%, in the third 13% and they decreased in the course of the illness more remarkably in children than in adults.

Metamyelocytes counted on the second day 18.6%. Then, they increased in the course of the illness and reached their maximum of 30.9% on the eighth day, decreasing thereafter. They appeared in the first week in the percentage of 24.9%, in the second of 26.3%, in the third of 19.6%. The increase and the decrease of metamyelocytes were more remarkable in children than in adults. On the other hand in the peripheral blood metamyelocytes counted 1.5%on the second day, 2.3% on the fifth day, 0.5% on the seventh day, but after the eighth day they disappeared from the peripheral blood. The appearance of metamyelocytes in the peripheral blood amounted in the first week to 1.2% and

in the second to 0.1%.

Rod-shaped leucocytes in the bone marrow decreased relatively at the onset of the illness, counting 11.3% on the second day, but they increased after that and they appeared on the seventh day in a percentage over 20%, and from the eleventh to the fourteenth day in the range of 24–25%, increasing on the twentieth day to 28.9%. The increase and the decrease of the rod-shaped leucocytes were more remarkable in children than in adults. In the peripheral blood they counted 34% on the second day but decreased in the later course. They amounted in the first week for 26.2%, in the second for 13.8% and in the third for 21.8%.

Segmented leucocytes decreased at the onset of the illness in the bone marrow and after the eleventh day returned to the normal.

Sum total of the neutrophil cells. In the bone marrow they decreased remarkably and the sum total of them fell to 70.4% on the second day but after the sixth day they increased and reached their maximum of 81.6% on the eighth day, redecreasing to the normal level after the third week. In the peripheral blood they increased strikingly at the onset of the illness showing a picture of symptomatic leucocytosis. After the sixth day they decreased gradually and reached their minimal value of 40% on the thirteenth day, recovering the normal level in the later course.

Eosinophil cells. Eosinophil cells in the bone marrow increased at the onset of the illness and showed 4.3% on the second day. They increased gradually and reached their maximum of 6.5% on the sixth day. After that they decreased gradually, but they showed a supernormal value throughout the entire course of the illness. In the peripheral blood they increased to 8% on the second day, that is, in the eruptive stage, but decreased gradually thereafter. They appeared in the blood in the first week in the percentage of 5.7%, in the second of 4.4%, in the third of 4.7%, while in the bone marrow in the first week the percentage was 6.6%, in the second 4.6% and in the third 3.3%.

Basophil cells. Basophil cells were seen in the low percentage of 0–0.08% in the fever stage but increased to 0.15% on about the twelfth day in the bone marrow, while in the peripheral blood they did not appear till the tenth day, after that they appeared first in the blood and showed a postinfectious basophilia.

Monocytes. Monocytes in the bone marrow increased slightly in the course of the illness, but in the blood they increased from the onset of the illness, showing a maximum of 8.3% on the eighth day. They appeared in the blood in the first week in the percentage of 5.8%, in the second of 6% and the third of 5.5%.

Lymphocytes. Lymphocytes in the bone marrow were in the count of 19.2% on the second day, after that they decreased relatively and reached the minimum of 7.9% on the eighth day. They appeared in the first week to the extent of 15.2%, in the second of 12.7%, in the third of 14.5% and after that they returned to the normal level. On the other hand they decreased in the blood from the onset of the illness. Lymphocytes in the blood counted in the first week 19.3%, in the second 33.1%, and in the third 28%. The lymphocytes

TABLE

Peripheral Blood Picture of

Day or week of illness			Day									
			2	4	5	6	7	8	9	10	11	12
No. of cases			1	7	6	9	5	3	2	2	4	2
R. B. G. (million)			3.90	4.45	4.31	4.22	4.22	4.37	4.37	4.00	4.29	4.04
Hgb. (%)			82.0	86.1	85.5	81.8	75.8	87.3	86.5	81.0	83.0	85.5
Color-index			1.05	0.97	0.99	0.96	1.02	0.98	1.01	1.01	1.02	1.03
W. B. G.			16800	13785	15383	16522	13640	21400	14550	17250	10000	8200
Leucocytes (%)	Neutrophils	Metamyeloc.	1.5	0.4	2.3	1.4	0.5	0	0	0	0.4	0
		Rod-shaped	34.0	28.8	34.1	21.8	19.2	14.7	11.8	14.3	19.1	8.8
		Segmented	40.5	43.2	36.3	37.8	41.7	45.2	45.0	41.3	39.6	37.8
		Total	76.0	72.6	72.7	61.0	61.4	59.8	56.8	55.5	59.1	45.8
	Eosinophils	Rod-shaped	1.50	1.78	1.00	1.11	1.20	0.83	0.25	0.25	0.50	0.5
		Segmented	6.50	4.92	4.00	6.50	5.10	5.33	2.75	3.75	4.25	5.5
		Total	8.00	6.70	5.00	7.61	6.30	6.16	3.00	4.00	4.75	6.0
	Basophils		0	0	0	0	0	0	0	0	0.12	0
	Monoc.		5.5	5.88	5.91	6.05	5.00	8.33	4.50	3.25	4.87	7.75
	Lymphoc.		10.0	14.5	15.3	23.0	26.0	24.5	35.3	36.0	30.5	40.5
	Plasma cells		0.50	0.35	1.16	1.61	1.2	1.16	0.5	1.25	0.62	0

were inversely proportionate to the neutrophil cells and showed higher percentages in children than in adults both in the bone marrow and the blood.

Reticuloendothelial system (Table I, II, III)

Lymphoid reticular cells increased slightly in the course of the illness in the bone marrow, while none appeared in the blood. *Plasma cells* in the bone marrow amounted for 1.2% on the second day and showed a maximal value of 3.5–3.8% on the seventh and eighth day. They averaged 2.5% in the first week, 1.9% in the second and 2.6% in the third. They appeared in lower percentages in children than in adults. In the blood they appeared on the second day only in the percentage of 0.5% but after that they increased and rose to 1.6% on the sixth day and to the maximal value of 3.5% on the thirteenth-day. In the blood the palsma cells were found, vice versa, more numerous in children than in adults. From this fact it seems that even if the plasma cells observed in the blood can be produced partly in the bone marrow, the mjority of them are produced extramedullarly. *Macrophages* appeared in the first week in the percentage of 0.03%, in the second of 0.01% and in the third of 0.03% only in the bone marrow. *Megakaryocytes* were seen only in the bone marrow and increased slightly in the first and the second week.

Bacteriological examination

III

the Scarlet Fever Cases

				Week			Week					
13	14	20	21	I	II	III	I		II		III	
1	1	2	1	28	15	3	Child 21	Adult 7	Child 12	Adult 3	Child 3	Adult 0
4.05	3.91	3.80	3.45	4.28	4.20	3.69	4.20	4.53	4.11	4.57	3.68	
93.0	73.0	76.0	75.0	82.6	80.5	75.6	80	88.5	83.3	95.3	75.6	
1.10	0·98	0.99	1.08	0.98	1.00	1.02	0.98	0.98	1.01	1.04	1.02	
8900	17700	13050	11800	15089	14040	12633	15185	14800	11050	9933	12633	
0	0	0	0	1.2	0.1	0	1.2	1.3	0.1	0	0	
8.5	8.0	29.0	7.5	26.2	13.8	21.8	24.3	31.6	13.2	16.2	21.8	
31.5	41.5	36.2	47.5	39.6	38.1	40.0	36.0	50.5	32.3	41.2	40.0	
40.0	49.5	65.3	55	66.9	52.0	61.8	61.5	83.4	45.6	57.3	61.8	
0.5	0	0.5	0	1.28	0.46	0.33	1.23	1.42	0.45	0.5	0.33	
2.5	2.5	1.75	5.5	5.32	4.13	3.00	5.34	5.21	4.08	4.33	3.00	
3.0	2.5	2.75	5.5	6.6	4.59	3.33	6.59	6.64	4.54	4.83	3.33	
0	0	0	2.5	0	0.06	0.83	0	0	0.08	0	0.83	
9.50	5.00	5.0	4.5	5.76	6.00	5.5	5.90	5.3	6.0	6.0	5.50	
43.5	40.0	25.5	33.0	19.30	33.06	28.0	22.5	9.7	34.3	31.3	28.0	
3.5	3.0	2.1	0	1.1	1.06	1.4	1.84	0	1.9	0.5	1.4	

Of all the 38 scarlet fever cases treated the pharyngeal mucus and bone marrows were cultured. Hemolytic streptococci were positive in the pharyngeal mucus of 33 cases (86.8%), but never in the bone marrow.

Comment

Both in the bone marrow and the peripheral blood the red cell count and the hemoglobin content decreased gradually in the course of scarlet fever. This anemia was caused by pathogenetic agent, appearing as a secondary anemia. The anemia was more remarkable in children than in adults.

The nucleated cells in the bone marrow stood at 490,000–60,000. They were more numerous in children than in adults, as Pachioli[4] and Kato[5] have reported. The leucocyte counts in the peripheral blood showed a remarkable symptomatic leucocytosis of 16,800 with a marked nuclear shift to the left on the second day, while in the bone marrow a decrease of reserved cells with a nuclear left shift was seen.

These findings indicated that the white blood cells flowed rapidly and actively out of the bone marrow into the peripheral blood. Then the

leucocytes in the peripheral blood slightly decreased for a while and increased until the eighth or tenth day but decreased gradually to the normal counts in the later course.

In the bone marrow picture there is no much change in appearance of myeloblasts throughout the entire course, as Leitner[6] has reported. Promyelocytes increased on the second day, especially remarkably in children, since the bone marrows of children are more sensitive and reactive against the impulse of pathogenetic agents than those of adults. Myelocytes showed a relatively high percentage on the second day as a result of mobilization of reserved cells, but soon after they decreased remarkably. On the sixth day they increased again and showed a picture of a myelocytic nuclear shift to the left, that is, a neutrophil myelocytic reaction, as Leitner[6], Révol and Chalier[7] and Samukawa[8] have reported. The abovementioned cells did not appear in the peripheral blood.

Metamyelocytes reached their maximal value of 30.9% in the bone marrow on the eighth day while in the blood they appeared to the amount of 1.5% on the second day, of 2.3% on the fifth day and of 0.5% on the seventh day, but after the eighth day never appeared again in the peripheral blood.

Rod-shaped leucocytes in the bone marrow decreased at the onset of the illness, soon increasing gradually and showing their maximal value of 24.4% on the eleventh day, decreasing then gradually to the normal value, while in the blood they increased remarkably and showed a symptomatic leucocytosis with a nuclear shift to the left at the beginning and gradually decreased to the normal in the later course, showing a remarkable decrease after the twelfth day.

Segmented leucocytes in the bone marrow decreased markedly at the beginning, but increased remarkably after the twelfth day. In the blood they appeared in the range of 31.5–47.5%.

The leucocyte count is under the control of neutrophils, therefore it can be understood that the leucopoietic function of the bone marrow is reflected in the change of neutrophils, i.e., promyelocytes showed their maximal value on the second day, myelocytes on the sixth day, metamyelocytes on the eighth day and the rod-shaped leucocytes on the eleventh day. Consequently a neutrophilia with increase in the various kinds of cells appeared, the increase beginning first in primitive cells and later gradually in more mature ones, as Fig. 1 shows. In short the bone marrow on about the second day can be called a promyelocytic marrow, on about the sixth day a myelocytic marrow, on about the eighth day a metamyelocytic marrow and on about the eleventh day a mononuclear leucocytic marrow.

Furthermore it is particularly noteworthy that the mechanism of the

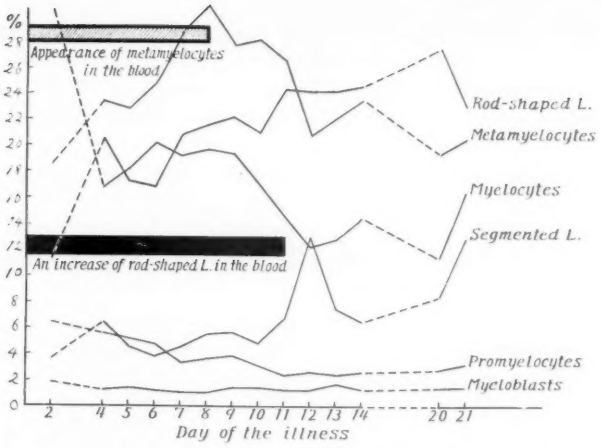

Fig. 1. Change of various kinds of the nucleated cells in the leucopoietic system in the bone marrow of leucocytosis cases.

outflow of the white blood cells into the blood is made clear in this serial examination. That is, metamyelocytes increased in the course of the illness and showed their maximum on the eighth day and then gradually decreased in the bone marrow, while on the other hand they appeared in the peripheral blood as long as they were increasing in the bone marrow, but after the eighth day when they decreased they appeared no longer in the blood notwithstanding the fact that they were in a high percentage in the bone marrow. In the same way, the rod-shaped leucocytes increased and showed their maximum on the eleventh day and after that decreased in the bone marrow, while in the blood they increased as a result of symptomatic leucocytosis at the onset of the illness. After that they decreased gradually but showed a high percentage in the blood as long as they were increasing in the bone marrow, but after the twelfth day they decreased rapidly in the blood.

This fact seems to indicate that the appearance of these younger cells in the blood does not mean they are in high percentages, but they are increasing actively in the bone marrow.

The mechanism of the outflow of younger cells into the peripheral blood can be understood easily on the basis of the falling gradient between the bone marrow and the blood. The gradient depends upon the bone marrow function on one hand and the peripheral demand on the other. At the onset of the illness the gradient becomes so steep, since the peripheral demand for the cells increases, that the younger cells flow actively out of the bone marrow into the blood, but thereafter the new proliferation in

the bone marrow becomes greater in order to comply with the peripheral demand. Therefore the gradient remains steep, but after the acme stage the peripheral demand decreases gradually, and accordingly the increase of the cells stops or they decrease in the bone marrow. Consequently, as the gradient becomes smaller, the younger cells disappear or decrease in the peripheral blood.

Eosinophils increased both in the bone marrow and blood at the onset of the illness.

Basophils decreased at the beginning in the bone marrow and disappeared from the peripheral blood, but in the course of the illness they increased.

Monocytes remained in low percentage in the bone marrow, showing a slight increase in the course of the illness, but increased remarkably in the blood, reaching a maximum of 8–9% in the second week, after that returning to the normal. Both in the bone marrow and the blood lymphocytes decreased strikingly in the stage when neutrophil leucocytes remarkably increased, and with the decrease of the latter relatively increased the former, so the percentages of lymphocytes were inversely proportionate to those of neutrophil leucocytes.

Reticuloendothelial cells increased in the bone marrow, decreasing in the course of the illness. Both in the bone marrow and the blood plasma cells increased from the onset of the illness, reaching their maximum on the fifth—the eighth days. Macrophages and megakaryocytes increased slightly in the bone marrow in the first and the second week.

Conclusions

The authors have observed 38 scarlet fever cases (28 children and 10 adults). In these cases 46 bone marrow specimens were examined, using as controls blood pictures obtained at the same time. The results were as follows.

1. In scarlet fever cases a secondary hyperchromatic anemia was observed in the course of the illness.

2. The nucleated cell count in the bone marrow increased in the acme of the illness and the leucocyte count increased strikingly in the blood at the onset of the illness, especially noticeably in children.

3. It is a very important finding in the bone marrow that a neutrophilia with increase in the various kinds of cells appeared, the increase being first observed in primitive cells and later gradually in more mature ones.

4. An increase in the outflow of younger cells from the bone marrow into the blood does not always mean that they are in high percentage in the bone marrow, but the increase is related to the potential gradient between

the bone marrow and the blood. An increase of the outflow of younger cells means that they are increasing in the bone marrow. Therefore they disappeared from the blood soon after the stop of their increase in the bone marrow, even if they were in high percentage in it.

5. It is evident that the bone marrow of children is more sensitive against impulse of pathogenetic agents than that of adults, and that the more is the cell primitive, the more sensitive is it to stimulation and the more easily falls it into degeneration.

6. Eosinophil leucocytes increased both in the bone marrow and the blood at the onset of the illness and decreased in the later course. Basophil leucocytes decreased at the onset but after that increased in the course of the illness. Monocytes remained in a low percentage in the bone marrow but decidedly increased in the blood in the second week. Lymphocytes decreased in the acme stage, so their percentage was inversely proportionate to that of neutrophil leucocytes in the bone marrow, and particularly in the blood. Plasma cells increased at the beginning of the illness, then decreased in the later course. Macrophages and megakaryocytes in the bone marrow increased slightly at the beginning of the disease.

7. Str. hemolyticus was cultured from the pharyngeal mucus in 86.8% of the cases, but never from the bone marrow and the blood.

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Studies on Leucocytosis and Leukopenia in Infectious Diseases

Report II

The Hematopoiesis of the Bone Marrow in Leucopenia

By

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In our studies on the hematopoiesis of the bone marrow in leucopenia we examined 11 typhoid fever cases. The bone marrows of all patients were observed regularly once 5–7 days by serial punctures following recovery, except in case of death. In every case from one to twelve bone marrow punctures were made, amounting to 45 punctures.

Experimental

Methods

In the same procedures as discussed in the previous report[1] 11 typhoid fever cases were observed. The diagnosis of these cases was established on the basis of positive findings of typhoid bacilli in the bone marrow cultures. They were selected from subjects not vaccinated for these several years, and treated in our hospital without administration of antibiotics such as chloromycetin in order to keep them in the natural course of the disease. The bone marrow pounctures were done regularly once 5–7 days, and the myelogram was compared with the hemogram, which was prepared on every other day.

Results

Normal myelogram

The normal myelogram was established on the basis of the average rate of 10 normal persons (6 male and 4 female). As Table 1 shows, rod-shaped and segmented leucocytes are in lower percentages in our myelogram than in Rohr's,[2] but there is no great difference between the two. The nucleated cell count in the bone marrow was 117,600 on the average.

Bone marrow picture and blood picture in typhoid fever

According to the stages of the illness, all the cases were divided into 4 groups of fever-rising, acme, fever-abating and reconvalescent stages. The pictures of

TABLE I

Bone Marrow Picture of 10 Normal Cases

				Max.—min.	Average value	Rohr
Reticulo-endothel	Lymphoid cells			4.0–1.0	2.5	6–1
	Plasma cells			4.4–0.8	2.5	2 } 8
	Macrophages			0.1–0	0–0.1	0
Erythro-poiesis	Erythroblasts					
	Basophil			5.2–1.2	3.0	5
	Polychromatic			32.1–13.8	20.0	9 } 30
	Oxyphil			8.1–1.5	4.4	16
Leuco- and lymphopoiesis	Myeloblasts			2.5–1.0	2.0	1
	Neutrophils	Myelocytes	Unmatured	3·8–1.2	2.6	4 } 10
			Half-matured	7.5–4.1	6.4	6
			Matured	13.0–8.9	11.2	7 } 16
			Metamyeloc.	14.8–9.8	12.2	9
		Rod-shaped		39.1–27.3	32.1	41 } 58
		Segmented		22.0–6.8	12.9	17
		Total			77.4	
	Basophils			0.5–0	0.2	0–1
	Eosinophils			7.8–2.6	4.7	4
	Monocytes			3.0–1.0	1.8	2
	Lymphocytes			20.3–9.2	14.3	11
Remarks	Nucleated cells in B.M.				117.600	
	R. B. C. in B. (million)				5.05	
	Hgb. (%)				99.8	
	Color-index				0.94	
	W. B. C. in B.				6900	
	Mitosis index					
	erythrop. system ‰				43.1	42
	leucop. system ‰				2.8	2.5

the bone marrow and the blood in each stage are shown in Table II and III.

The nucleated cell counts in the bone marrow decreased in the course of the illness, showing a noticeable decrease from the latter half of the acme, and reached the minimum of 68,000 on the average in the fever-abating stage. They increased thereafter gradually to the normal. In the peripheral blood leucocytes remained normal or increased slightly over the normal at the onset of the illness, but decreased strikingly from the latter half of the acme, reaching their minimum of 4,900. They returned gradually to the normal after the reconvalescence.

Reticuloendothelial system. There was no much change in lymphoid reticular cells, but plasma cells increased from the onset of the illness, reaching their maximum of 3.1% in the acme (the third week of the illness). These cells increased remarkably in the severe, particularly in the fatal cases. In the peripheral blood plasma cells increased parallel to those in the bone marrow. Macrophages remained in a low percentage but slightly increased at the onset of the illness.

Erythropoietic system (Table II). Erythroblasts decreased from the beginning

TABLE II

Bone Marrow Picture in Every Stage of Typhoid Fever

Fever stage				Fever-rising	Acme		Fever-abating	Reconvalescence	
					First half	Latter half			
No. of cases				1	9	8	9	9	9
Reticulo-endothel			Lymphoid cells	2.4	2.2	3.1	1.0	0.9	1.0
			Plasma cells	2.4	3.2	5.1	2.6	2.0	1.6
			Macrophages	0.1	0.1	0	0.1	0	0
			Total	4.8	5.4	8.2	3.6	2.9	2.6
Erythro-poiesis	Erythroblasts		Basophil	4.2	4.1	7.1	6.5	5.7	5.7
			Polychromatic	7.6	11.6	23.4	25.6	21.4	19.5
			Oxyphil	0.6	1.6	4.0	3.1	3.7	2.9
			Total	12.4	17.3	34.5	35.2	30.8	28.1
Leuco- and lympho-poiesis	Myeloblasts			3.6	3.5	3.3	2.9	3.1	2.7
	Neutrophils	Myelocytes	Unmatured	8.7	4.6	5.8	4.6	5.4	4.6
			Half-matured	18.4	10.3	11.2	7.6	7.8	5.9
			Matured	14.3	12.4	12.3	10.9	12.4	12.3
			Metamyelocytes	12.3	16.3	17.9	14.7	17.0	15.2
			Rod-shaped	16.0	26.9	21.2	27.6	29.3	28.8
			Segmented	1.3	5.1	4.7	5.0	3.6	5.6
			Total	71.0	75.6	70.1	70.4	75.5	72.4
	Basophils			0.2	0	0.2	0.2	0.4	0.2
	Eosinophils			5.1	4.6	3.1	2.5	3.9	5.5
	Monocytes			0.6	1.1	1.1	1.1	0.9	1.4
	Lymphocytes			19.5	15.2	19.5	22.8	16.1	20.1
Megakaryocytes				−	++	−	−	++	+
Nucleated cells in B. M.				272000	122000	89000	68000	86000	91000
M.I.	Erythropoietic ‰			44.2	40.4	43.6	38.4	39.0	40.4
	Leucopoietic ‰			2.6	2.2	1.8	2.0	3.4	3.0

and markedly in the first half of the acme, after that they increased to the normal, showing a percentage of 35.2% in the fever-abating stage. In the peripheral blood the red cell count decreased from the onset of the illness, showing the minimum of 3.93 mill. in the latter half of the acme, the minimal hemoglobin content was 66.7% and the color-index showed 0.84 in the fever-abating stage. They increased gradually to the normal in the later course.

Leuco- and lymphopoietic System (Table II and III). Myeloblasts increased relatively in 3.6–3.5% in the fever-rising stage and they showed supernormal values throughout the entire course of the illness.

TABLE III

Blood Picture in Every Stage of Typhoid Fever

Fever stage			Fever-rising	Acme		Fever-abating	Reconvalescence	
				First half	Latter half			
No. of cases			1	9	8	9	9	9
Peripheral blood	Neutrophils	Matured myelocytes	0.1	0.1	0	0	0	0
		Metamyel.	4.4	2.3	0.8	0	0	0
		Rod-shaped	63.6	47.5	39.2	29.9	25.0	25.4
		Segmented	16.0	21.2	18.7	14.6	11.0	16.3
	Basophils		0	0.1	0.1	0.4	0.5	0.5
	Eosinophils		0	0.1	0.2	1.4	2.3	3.3
	Monocytes		2.0	3.5	3.2	5.1	5.6	3.9
	Lymphocytes		14.0	23.9	36.0	46.6	55.2	49.8
	Plasma cells		0	1.0	1.5	0.6	0.3	0.5
	W.-B. C.		9200	7700	5000	4900	5500	6600
	R. B. C. (million)		4.01	4.22	3.93	3.97	3.93	4.04
	Hgb. (%)		78.0	78.0	70.87	66.66	69.31	72.55
	Color-index		0.77	0.92	0.89	0.84	0.88	0.90

Neutrophil cells. Unmatured and half-matured myelocytes increased relatively, and showed the percentage of 27.1% in the fever-rising stage and 17% in the latter half of the acme. From the fever-abating stage they turned to a decrease, reaching the about normal percentage of 10.5% in the second week after the termination of fever. These cells remained over the normal throughout the entire course of the illness.

Matured myelocytes and metamyelocytes increased in the entire course of the illness. The percentage of these cells was 28.7% in the first half of the acme (the second week) and reached their maximum of 30.2% in its latter half (the third week), after that decreasing to the normal, but only in 3 cases, they did not decrease to the normal in the second week after the termination of fever. In the peripheral blood matured myelocytes appeared in the percentage of 0.1%

in the first half of the acme, and metamyelocytes were seen from the onset of the illness to the latter half of the acme (Fig. 1).

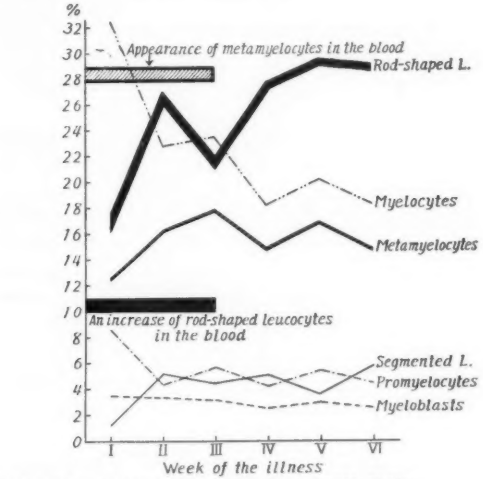

Fig. 1. Change of various kinds of the nucleated cells in the leucopoietic system in the bone marrow of leucopenia cases.

Rod-shaped and Segmented leucocytes in the bone marrow decreased strikingly from the onset of the illness, the decrease being first the result of mobilization of these cells into the peripheral blood. They decreased to 17.3% on the second day. After that they rose to 32% in the first half of the acme, and decreased to the minimum of 25.9% in it's latter half. From the fever-abating stage they gradually increased, but they were in a remarkably low percentage throughout the entire course.

Of these 11 cases there were only 2 cases, in which they increased to the normal percentage in the second week after the termination of fever.

Observing all neutrophil cells in the various stages of maturation, we found that the neutrophil cells in the bone marrow decreased as a result of mobilization into the peripheral blood at the onset of the illness. On the other hand, cell proliferation gradually fell down and consequently the neutrophil cells decreased, reaching their minimum of 70.4% in the fever-abating stage. Furthermore in this stage the absolute count of the nucleated cells in the bone marrow decreased to a half or two fifths of that at the beginning of the disease, so we could know the bone marrow in the fever-abating stage of typhoid fever was very poor in neutrophil cells. In the peripheral blood a symptomatic neutrophilia with a nuclear shift to the left appeared as a result of mobilization of the neutrophil cells from the bone marrow at the beginning, but in the later course of the illness

they decreased and showed a striking decrease in the latter half of the acme and the fever-abating stage. They increased gradually to the normal after the reconvalescence.

Basophil cells in the bone marrow decreased from the onset of the illness and increased in the reconvalescence. The decrease and the increase of these cells in the bone marrow was followed by a similar change in the blood about 4–7 days after wards.

Eosinophil cells appeared in the bone marrow in the percentage of 5.1–4.6% down to the first half of the acme, decreasing in the later course of the illness. They reached their minimum of 2.5% in the fever-abating stage, and showed an eosinophilia of 5.7% and more from the second week of the reconvalescence. These cells in the blood decreased from the onset of the illness and in the majority of cases they disappeared from the blood in the acme. Appearing first in the fever-abating stage, they increased to an postinfective eosinophilia in the blood several days later than in the bone marrow.

Monocytes remained in the low percentage of 0.9–1.4% throughout the entire course of the illness, but on the contrary they increased remarkably in the blood from the fever-abating stage, attaining the normal level in the reconvalescence.

Lymphocytes increased relatively over the normal throughout the entire course of the illness, showing remarkably that they were inversely proportionate to neutrophil cells in the percentage of the myelogram. Lymphocytes appeared in the bone marrow in the fever-rising stage in the percentage of 19.5%, in the first-half of the acme of 15.2%, in its latter half of 19.5%, and reached the maximum of 22.8% in the fever-abating stage, decreasing gradually in the later course of the illness. The severer the clinical features were, the more striking was the relative lymphocytosis. In the periphery a leucopenia with retative lymphocytosis were seen.

Mitosis in the bone marrow

The mitosis index in the erythropoietic system stood at about the normal of 44.2‰ at the beginning of the disease, and then dropped below normal, reaching the minimal value of 38.4‰ in the fever-abating stage. Thereafter it rose to 40.4‰ in the second week after the termination of fever. The mitosis index in the leucopoietic system dropped from the onset of the illness, reached their minimum of 1.8‰ in the latter half of the acme, but recovered about the normal value in the second week in the reconvalescence.

Furthermore 2 cases are shown as examples: one with typical, another with atypical course of the illness.

Case I. O.S. girl, aged 7, severe typhoid with typical course, was admitted to our hospital on the second day of the illness; the termination of fever was on the 22nd day; complicated with bronchopneumonia at the onset of the illness (Table IV. a, b). The nucleated cell count was 272,000 on the fourth day, 61,000 in the acme and the fever-abating stage, 102,000 in the second week of the reconvalescence. The leucocyte count in the blood was 10,200 on the second day, 10,700 in the fourth day of the illness and then decreased to the level of

Errata

A Case of Cbngenital Leukemia The 6th Case Reported in Japan

By

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In our treatise of the above shown title, this Journal 1957, Vol. 66, p. 307-316, we desire to make the following corrections: —

In Table I, p. 309,	12th line	read Myelocytes for	Myeloblasts,
"	13rd "	" Metamyelocytes "	Metamyeloblasts,
in Table II, p. 310,	10th "	" Myelocytes "	Myelocites,
and p. 314,	17th line	" Japanese "	Fapanese.

TABLE IV. a

Bone Marrow Picture. Case of O. S. (7 years old ♀)

	Fever stage			Fever-rising	Acme		Fever-abating	Reconvalescence	
	Week of the illness (Day of the illness)			$I_{(4)}$	$II_{(9)}$	$III_{(15)}$	$IV_{(22)}$	$V_{(29)}$	$V_{(36)}$
Reticulo-endothel	Lymphoid cells			2.4	2.5	2.6	0.4	0.6	0.4
	Plasma cells			2.4	1.7	1.7	1.6	0.7	0.1
	Macrophages			0	0	0	0	0	0
Erythro-poiesis	Erythro-blasts	Basophil.		4.2	4.5	9.1	18.6	11.0	8.2
		Polychromatic.		7.6	8.4	18.1	49.4	27.0	13.2
		Oxyphil.		0.6	0.2	3.6	5.8	8.2	3.0
Lenco- and lymphopoiesis	Myeloblasts			3.6	3.4	2.3	1.8	2.4	2.0
	Neurtrophils	Myelocytes	Unmatured	8.7	7.0	5.5	5.6	5.9	6.7
			Half-matured	18.4	12.4	5.6	5.7	5.0	7.7
			Matured	14.3	16.9	9.3	14.8	15.2	16.2
			Metamyel.	12.3	13.1	17.8	18.6	18.1	19.8
		Rod-shaped		16.0	22.2	24.1	26.0	26.1	27.1
		Segmented		1.3	3.1	7.6	3.8	4.0	2.4
		Total		70.0	74.7	69.6	74.7	74.0	79.9
	Basophils			0.2	0	0.3	0.5	0.3	0.2
	Eosinophils			5.1	5.1	5.0	2.5	4.1	7.7
	Monocytes			0.6	0.8	2.2	0.6	0.8	0.7
	Lymphocytes			19.5	16.0	20.3	20.1	18.0	9.6
Remarks	Nucleated cells in B. M.			272000	89000	61000	61000	83000	102000
	R. B. C. in B. (million)			4.01	3.98	3.88	4.07	3.98	4.00
	Hgb. (%)			78	72	60	62	64	69
	Color-index			0.77	0.92	0.78	0.77	0.82	0.86
	W. B. C.			9200	10600	6000	3100	2900	5800
	M. I.	Erythropoietic ‰		44.2	38.2	36.4	40.0	42.0	42.0
		Leucopoietic ‰		3.0	2.8	2.0	1.6	4.0	2.4
	Degeneration			(±)	(±)	(±)	(+)	(+)	(+)

2,200–3,200 in the fever-abating stage and reconvalescence. Lymphoid reticular cells increased at the beginning of the disease, decreasing in the later course to the normal in the bone marrow. Plasma cells increased to 2.4% at the beginning, but then decreased gradually to the normal. They appeared first in the blood in the acme, and reached the maximum of 1.8% in the reconvalescence. Erythroblasts decreased from the onset to the acme and then strikingly increased to 63.8%, but they decreased to the normal of 24.5% in the second week of the

TABLE IV. b

Peripheral Blood Picture. O. S. (7 years old ♀)

Fever stage		Fever-rising		Acme							Fever-abating		Reconvalescence				
Day of the illness		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
Neutrophils	Metamyelocytes	4.4	6.8	2.8	4.8	5.2	2.0	1.6		0.4							
	Rod-shaped	63.6	55.2	59.6	58.8	44.8	56.4	54.4	40.0	41.6	38.0	39.6	28.8	19.6	18.4	29.6	26.0
	Segmented II	15.6	17.6	20.8	16.8	10.8	17.6	20.8	16.0	18.4	18.0	16.4	11.2	6.4	6.0	10.4	10.6
	" III	0.4	0.8	0.8	1.6		1.6	1.6	1.0	1.2	2.4	1.2	1.6	0.8	0.8	0.8	0.8
	" IV		0.4					0.8									
	Total	84.0	80.8	84.0	82.0	60.8	77.6	79.2	57.0	61.6	58.4	55.2	41.6	26.8	25.2	38.8	37.4
Basophils											0.8	0.8	0.6	0.8	0.4	0.8	0.8
Eosinophils														0.8	2.4	1.2	1.0
Monocytes		2.0	5.6	4.4	2.4	3.2	5.6	1.2	4.5	5.2	3.2	6.4	6.8	7.2	4.8	5.6	1.0
Lymphocytes		14.0	17.6	10.8	14.4	36.0	16.0	18.8	37.5	32.8	26.0	36.8	50.4	62.6	62.0	52.0	61.4
Plasma cells				0.8	1.2		0.8	0.8	1.0	0.4	1.6	0.8	1.2	1.8	1.2	1.6	0.4
W. B. C. (thousand)		10.2	10.7	9.6	10.6	8.4	7.1	7.4	6.6	3.2	6.3	3.1	4.5	2.2	2.9	3.5	5.8

reconvalescence. In the blood the red cell count showed a slight decrease in the acme. In the leucopoietic system of the bone marrow myeloblasts increased, and unmatured and half-matured myelocytes increased remarkably in the acme. Matured myelocytes and metamyelocytes increased to 26.6% on the fourth day, and further increased in the fever-abating stage and the reconvalescence. Metamyelocytes appeared in the blood at the onset of the illness and reached their maximum of 6.8% in the fourth day, but they appeared no longer in the blood after the twentieth day. Rod-shaped and segmented neutrophil leucocytes in the bone marrow decreased throughout the entire course of the illness. Their extreme decrease in the blood to 25.2% was seen in the first week of the reconvalescence.

Basophil cells decreased in the acme, and then increased over the normal of 0.5% in the fever-abating stage, returning to the normal in the later course of the illness. In the blood a basophilia was seen in the fever-abating stage and the reconvalescence. Eosinophil cells remained in the bone marrow on the normal level of 5% till the acme and decreased to 2.5% in the fever-abating stage, but then increased again, showing an eosinophilia of 7.7% in the reconvalescence. These disappeared from the blood at the onset of the illness, but

appeared first in the blood in the reconvalescence. Monocytes remained in a subnormal range in the bone marrow, but in the blood increased and showed maximal rate of 7.2% in the reconvalescence. Lymphocytes in the bone marrow increased relatively from the onset throughout the entire course of the illness, and rose to 20.3% in the acme and the fever-abating stage. A relative lymphocytosis appeared throughout the entire course, showing a maximum of 62.6% in the blood in the reconvalescence. In the leucopoietic system the mitosis index dropped markedly in the acme and the fever-abating stage, showing the minimal value of 1.6‰.

Case II. K. T., School girl, aged 18, severe typhoid with atypical course, was admitted to our hispital on the thirteenth day of the illness. The termination of fever was on the 21st day, the first recurrence occurred on the 52nd day, its termination on the 68th day, the second recurrence occurred on the 86th day complicated with intestinal bleeding and pleuritis exsudativa typhosa. At the moment of this complication typhoid bacilli were cultured from the pleural exsudate. The termination of fever in the second recurrence was on the 94th day. She was discharged on the 116th day (Table V. a. b.).

The nucleated cell count in the bone marrow was about normal in the fever-rising stage, decreased strikingly in the fever-abating stage and the reconvalescence. The white cell count in the blood ran parallel to those in the bone marrow about several days afterwards. Lymphoid reticular cells and plasma cells increased at the acme. Erythroblasts decreased remarkably in the acme, increasing in the later course of the illness. Their increase and decrease in the bone marrow was followed by similar changes in the blood 10–15 days afterwards. The red cell count decreased to the minimum of 2.01 millions in the fever-abating stage. Myeloblasts increased in the bone marrow throughout the entire course of the illness, especially remarkable in the fever-abating stage. Unmatured, half-mutured and matured myelocytes and metamyelocytes increased during the fever stage, showing a marked increase in the acme in the bone marrow. In the blood matured and meta-myelocytes appeared at the peak of the illness. Rod-shaped and segmented leucocytes decreased throughout the entire course of the illness, showing a most remarkable decrease in the latter half of the illness. The increase and the decrease of their percentages in the bone marrow appeared as those in the blood 4–7 days afterwards.

As mentioned above the count of the neutrophil cells in the bone marrow decreased from the first half of the acme, reached its minimum during the latter half of the acme and the fever-abating stage, showing some irregularities and less sensitivity in the repeated recurrences. In the bone marrow basophil cells decreased in the acme and the fever-abating stage, increased in the reconvalescence, while they disappeared from the blood during the febrile period, increasing in the reconvalescence. Eosinophil cells showed a similar change as basophil cells in the bone marrow in the fever stage and increased after the reconvalescence. In the bone marrow monocytes presented nothing characteristic in change but in the blood they increased and reached their maximal height of 6.8% toward the fever-abating stage. The percentage of lymphocytes was inversely proportionate to

TABLE

Bone Marrow Picture. Case of K. S.

Fever stage				Acme		Remittent fever	Fever-abating
Week of the illness				II	III	IV	V
Reticulo-endothel			Lymphoid cells	1.3	3.1	1.3	1.1
			Plasma cells	5.2	7.0	4.1	2.9
			Macrophages	0	0	0	0
Erythro-poiesis	Erythro-blasts		Basophil	1.7	3.4	2.1	4.1
			Polychromatic	14.7	13.5	16.5	16.1
			Oxyphil	2.6	2.8	2.2	4.2
Leuco- and lymphopoiesis			Myeloblasts	4.0	3.9	3.4	2.8
	Neutrophils	Myelocytes	Unmatured	3.9	10.8	6.8	10.0
			Half-matured	8.0	12.9	8.8	11.0
			Matured	9.6	14.2	7.2	16.1
			Metamyel.	12.8	11.8	12.4	16.5
			Rod-shaped	36.1	15.3	32.5	21.1
			Segmented	9.5	2.6	2.6	2.5
			Total	79.5	67.6	73.9	77.2
			Basophils	0.1	0.5	0.1	0.2
			Eosinophils	0.6	2.2	1.6	0.3
			Monocytes	2.2	1.0	0.9	0.4
			Lymphocytes	13.2	24.8	20.1	19.1
Remarks			Nucleated cells in B.M.	249000	86000	78000	82000
			R.B.C. in B. (Mill.)	3.40	2.55	2.24	2.01
			Hgb. (%)	62	55	36	34
			Color-index	0.9	1.1	0.81	0.85
			W.B.C.	10200	6400	3400	6000
			M. I. Erythropoietic (‰)	34	38	38	36
			M. I. Leucopoietic (‰)	2.0	1.6	2.2	2.0
			Degeneration	(+)	(+)	(+)	(+)

that of neutrophil cells both in the bone marrow and the blood. Both in the erythropoietic and leucopoietic systems the mitosis decreased in the acme stage.

Summary and Comments

In the leucopenia of typhoid fever a nuclear shift to the left in the bone marrow was seen at the onset of the illness as a result of outflow of reserved cells into the peripheral blood, temporarily showing a symptomatic leucocytosis in the periphery. But in the later course of the illness mitosis in the bone marrow dropped down; besides, demand from the priphery increased gradually, so the matured cells in the bone marrow were mobilized

V. a
(18 years old ♀)

Fever free		I relapse			II relapse		
		Acme	Remittant fever	Fever free	Acme	Reconvalescence	
VI	VII	VIII	IX	X	XIII	XIV	XV
0.2	0.3	1.1	0.7	0.9	0.9	0.6	1.7
1.2	2.2	2.0	2.6	3.8	3.6	2.8	1.2
0	0	0	0	0	0	0	0
4.2	5.1	8.1	2.6	8.9	6.7	7.2	5.5
19.0	20.7	7.7	9.7	19.3	21.8	23.1	26.9
5.8	5.1	3.5	3.1	6.3	2.9	1.5	2.6
1.2	3.9	4.9	3.4	5.0	2.9	4.8	2.7
7.5	4.2	15.4	5.5	5.2	3.6	8.0	5.0
7.0	4.9	6.7	10.3	5.6	7.3	11.1	8.2
12.4	12.8	16.0	15.8	12.5	12.3	15.7	10.6
18.1	12.8	15.8	20.6	16.5	17.3	16.8	14.3
37.9	38.9	17.0	22.4	28.1	27.3	32.7	35.4
5.7	7.2	1.2	6.2	4.4	7.4	1.7	3.8
88.6	70.6	82.1	80.8	72.3	79.1	86.0	77.3
0	0.1	0	0	0	0.5	0.2	0.1
0.1	1.3	4.2	1.7	1.1	4.1	2.0	3.0
0.2	1.0	0.2	0.8	0.4	1.6	0.2	0.9
9.4	22.9	8.6	13.3	21.2	15.8	6.8	15.0
166000	53000	155000	65000	47000	120000	381000	391000
2.34	2.24	2.38	2.08	2.02	2.63	4.32	3.52
46	41	47	44	40	45	79	65
1.0	0.9	1.02	1.1	1.0	0.86	0.91	0.92
6500	6300	6400	7800	7300	5200	7600	7600
34	36	42	38	36	36	30	30
2.0	2.2	3.0	2.0	3.0	3.0	2.0	2.0
(++)	(+)	(++)	(++)	(++)	(+)	(±)	(±)

to the peripheral blood and consequently they decreased in the bone marrow, showing an extreme decrease of the nucleated cells, especially of rod-shaped and segmented leucocytes in the bone marrow in the latter half of the acme and the fever-abating stage. In this stage a nuclear shift to the left was obvious and the nucleated cell count decreased to a half or two-fifths of the normal count in the bone marrow. Concerning the decrease of the nucleated cells in the bone marrow of typhoid fever Naegeli,[3] Shilling,[4] Barta,[5] Galinowski,[6] Samukawa[7] and Kato[8] have reported. Storti and Filippi[9] called the bone marrow of typhoid cases "a marrow poor in cells." The reticuloendothelial cells increased in the

TABLE V. b

Peripheral Blood Picture. K. S. (18 years old ♀)

Fever period		Acme					Remittent fever				Fever-abating			Fever free							Acme			
Day of the illness		13	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45	47	49	51	53	55	57
Neutrophils	Matured myeloc.	0.6	-					0.4	0.2					0.2								0.4	0.4	1.0
	Metamyeloc.	0.4				1.2		1.6			0.4	0.8	0.4	0.6		0.4					0.4	1.6	5.8	5.8
	Rod-shaped	40.4	32.4	22.2	28.0	43.2	30.8	37.2	29.2	35.2	34.4	26.4	26.0	27.6	29.2	34.4	22.0	28.0	35.6	37.2	41.2	53.8	58.8	61.0
	Segmented II	34.2	40.4	25.4	25.0	21.4	24.8	28.8	25.2	21.6	21.2	19.2	20.8	13.6	14.4	14.0	14.8	18.0	21.6	22.0	18.4	7.2	6.6	15.4
	III	9.6	8.8	15.6	6.0	3.6	5.2	5.2	8.0	4.8	5.2	6.0	3.6	2.8	2.0	2.0	2.8	3.0	3.2	3.2	2.8			1.6
	IV			0.6		0.8	2.6		0.8	0.4	0.8	0.4	0.4	0.4	0.8			2.0	0.4					
	Total	85.2	81.6	63.8	59.0	70.2	63.4	71.2	63.4	62.0	62.0	52.8	51.2	45.2	46.4	50.8	38.6	51.0	60.8	62.4	62.8	63.0	71.6	84.0
Basophils											0.4			0.8			0.4							
Eosinophils				0.4			0.4								1.2	0.4	0.4	0	1.2				0.6	
Monocytes		2.4	0.8	0.4		0.8	2.4	2.8	1.6	2.4	4.4	3.2	2.8	5.2	2.4	3.2	4.4	1.0	1.6	0.8	0.6	1.0	0.8	2.0
Lymphocytes		11.6	17.2	35.4	41.0	28.4	33.4	25.6	35.0	35.6	33.2	43.6	45.2	48.0	50.0	45.6	56.2	48.0	36.0	35.6	36.6	36.0	27.0	14.0
Plasma cells		0.8	0.4			0.6	0.4	0.4			0.4		0.8	0.8					0	1.2	0.2			
W.B.C. (thousand)		10.2	8.2	6.0	7.8	6.4	3.2	4.2	3.4	6.4	6.0	5.4	6.0	6.3	7.2	6.5	8.3	6.2	4.9	6.3	5.0	4.7	6.4	4.5

I Relapse												II Relapse									
Remittent fever				Fever free								Acme				Reconvalescence					
59	61	63	65	67	69	71	73	75	77	79	81	83	85	87	89	91	93	95	97	99	101	103
0.4																						
	0.4	0.4														0.5	2.8					
51.6	42.4	36.0	29.2	20.0	20.8	19.8	14.0	25.8	14.4	21.6	33.2	33.2	21.6	33.2	23.0	43.0	37.6	32.0	17.0	16.0	8.5	17.0
25.2	27.2	20.0	17.2	14.2	16.4	16.4	7.2	11.8	10.0	18.4	15.2	10.8	18.4	15.2	11.0	5.5	4.8	10.0	13.0	12.0	10.5	15.0
4.4	4.0	1.2	3.2	5.6	4.0	3.0	0.4	1.8	2.8	2.8	2.4	0.6	2.8	2.4	0.5		0.4	1.2	1.5	1.5	1.5	1.0
	0.8			1.2							0.4			0.4						1.0		
81.6	74.8	57.6	49.6	41.0	41.2	39.2	21.6	39.4	27.2	42.8	51.2	44.6	42.8	51.2	44.5	49.0	45.6	43.2	31.5	30.5	20.5	33.0
							0.4			0.4	0.4	0.6	0.4	0.4	0.5	0.5	0.4				0.5	
		0.4	1.6	1.2	0.8	1.0	2.8	10.8	6.4	7.6	0.4		7.6	0.4			0.4	1.2	1.5	3.5	0.5	2.5
3.6	2.0	6.8	6.2	4.4	6.4	4.6	3.2	5.0	1.2	5.2	2.0	2.4	5.2	2.0	2.5	6.0	4.4	4.4	4.0	6.0	3.5	4.0
19.4	22.8	35.2	42.2	53.2	50.4	55.2	71.6	44.8	65.2	44.0	46.0	51.6	44.0	46.0	51.5	54.5	47.6	49.6	62.0	60.0	75.0	60.0
0.4	0.4		0.4	0.2	1.2		0.4					0.8			1.0	0.5	1.6	1.6	1.0			0.5
7.8	4.1	4.9	7.3	4.6	4.0	3.8	5.0	5.2	4.4	5.2	6.2	4.2	3.4	4.4	4.8	6.4	7.6	6.8	7.0	8.6	7.3	7.6

fever stage and showed a maximal value in the acme, increasing markedly in severe cases. In the peripheral blood their increase or decrease was seen a little while later.

In the erythropoietic system erythroblasts decreased at the onset but increased in the acme and reached the maximum of 35.2%. Their increase or decrease in the bone marrow was followed by a similar change in the blood 10–15 days afterwards.

In the leucopoietic system myeloblasts increased in every case throughout the entire course of the illness, as Naegeli,[3] Barta[5] and Murase[10] have reported, but their increase was not so large as Naegeli says. Unmatured and half-matured myelocytes increased relatively, showing their peak in the acme stage. Matured myelocytes and metamyelocytes increased relatively throughout the entire course and appeared in the blood in the fever-rising and acme stage, but they disappeared from the blood after the fever-abating stage. On the contrary rod-shaped and segmented leucocytes in the bone marrow decreased from the beginning of the illness and reached the minimal level in the latter half of the acme (the third week), then they increased gradually, but they remained yet subnormal even in the second week of the reconvalescence. Their decrease in the bone marrow was followed by a decrease in the blood 4–5 days afterwards. They showed the most remarkable decrease with a nuclear shift to the left in the blood during the latter half of the acme and the fever-abating stage.

Both eosinophil and basophil leucocytes in the bone marrow decreased from the onset of the illness and reached their minimal level in the latter half of the acme and the fever-abating stage, then they increased gradually, showing an eosinophilia or a basophilia in the reconvalescence. In the peripheral blood a postinfective eosinophilia or basophilia appeared several days later than in the bone marrow.

Monocytes showed a slight increase in the bone marrow after the fever-abating stage even though they were in a low percentage, but a remarkable increase was seen in the blood during the same stage. The percentage of lymphocytes was inversely proportionate to that of neutrophil leucocytes, as many authors have reported, in the bone marrow. In the peripheral blood a remarkable, relative lymphocytosis was seen especially in the latter half of the illness.

In the case of leucopenia it is a noteworthy that the mechanism of outflow of the white blood cells into the blood is made clear in this serial examination, that is, metamyelocytes appeared and rod-shaped leucocytes increased in the peripheral blood as long as the white blood cells were decreasing in the blood (untill the end of the third week of the illness) as Fig. I shows.

Taken as a whole, the genetic mechanism of leucopenia in typhoid

fever is as follows:

In the leucopenia of typhoid fever the reserved cells standing in the right in the bone marrow are mobilized into the peripheral blood at the onset of the illness. Consequently there appeared a symptomatic nuclear shift to the left with a decrease of the nucleated cells in the bone marrow and a temporarily symptomatic leucocytosis with a nuclear shift to the left in the blood. Then the mitosis dropped gradually, showing the minimal value, the minimal nucleated cell count and a striking nuclear shift to the left in the bone marrow during the latter half of the acme and the fever-abating stage. In the reconvalescence the mitosis rose to about normal. The drop of the mitosis centered upon the unmatured and half-matured myelocytes at the beginning, on the matured myelocytes in the peak stage and mitosis rose gradually from the left to the right in the bone marrow in the later course.

The mitosis index dropped decidedly in typhoid, moderately in paratyphoid A and K (caused by Salmonella Sendai), and mildly in paratyphoid B cases, so in leucopenia the mitosis index dropped proportionate to the speciality and quality of the pathogenetic agents. Both in leucocytosis and leucopenia there is not much difference in the maturation, outflow into the circulating blood and destruction of the white blood cells.

Conclusions

The authors have observed 11 cases of typhoid fever and studied the hematopoiesis in the bone marrow of leucopenia. The bone marrow specimens of all the patients were obtained regularly once in 5–7 days by serial punctures following recovery, and the myeloid picture was compared with the blood picture which were prepared on every other day. The results were as follows.

1. The nucleated cell count in the bone marrow decreased gradually in the course of the illness, showing a decided decrease in the latter half of the acme and the fever-abating stage, then they increased to the normal in the reconvalescence. The nucleated cell count in the bone marrow was parallel to the leucocyte count in the blood.

2. The reticuloendothelial cells increased in the first half of the illness, then decreased to the normal in the bone marrow.

3. The erythroblasts decreased from the beginning, increasing gradually to the normal after the peak stage. The erythrocytes showed the same change in the peripheral blood 10–15 days afterwards.

4. In the leucopoietic system of the bone marrow myeloblasts increased relatively in the entire course of the illness, showing a remarkable increase in the acme stage.

5. The neutrophil cells in the bone marrow presented characteristics.

The rod-shaped and the segmented neutrophil leucocytes decreased strikingly in the entire course, and consequently a decidedly relative increase of unmatured, half-matured and matured myelocytes and metamyelocytes was observed, particularly in the acme and fever abating stage, when a nuclear shift to the left and a remarkable neutropenia with relative lymphocytosis was seen in the pripheral blood.

6. The basophil and the eosinophil cells in the bone marrow decreased from the beginning of the illness and showed their minimal value in the acme, when in the majority of cases they disappeared from the blood, but after that they increased in the bone marrow and appeared again in the blood, showing a postinfective basophilia and eosinophilia in the periphery.

7. The lymphocytes increased relatively throughout the entire course in the bone marrow, particularly showing a relative lymphocytosis in the acme and fever-abating stage, when a remarkable leucopenia with relative lymphocytosis was seen in the blood.

8. The mitosis index in the leucopoietic system in the bone marrow dropped in the course of the illness and showed its minimal value in the peak stage. The drop of the mitosis centered first upon primitive cells and later gradually on more mature ones. After the peak stage the mitosis rose gradually and showed about the normal value after the reconvalescence.

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Electromyographic Study of Patterns of Activity of the Abdominal Wall Muscles in Man

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Shiratori and others[1] in our clinic have recently made electromyographic studies, by means of needle electrodes, on the abdominal wall muscles in healthy humans. They made also some experiments for comparing the results obtained with needle electrodes with those by surface electrodes. Following them, I studied action potentials of the abdominal wall muscles in 50 healthy humans at rest and during various movements in the dorsal, standing, and other postures, with the results to be reported here, which have served to elucidate patterns of the abdominal wall muscles in various postures and during various movements and throw some light upon the problem of the activity of the abdominal wall.

Experimental

Methods

Placing of electrodes and conditions under which leads were taken. As shown in Figure 1, electrodes were placed at 10 positions, five on each side of the midline, on the skin above the upper, middle, and lower parts of the recti and above the external and internal obliques. The electrodes used were so-called plate electrodes, circular with a diameter of 0.6 cm. and with the middle part projecting upward.

Bipolar lead was taken with a pair of electrodes fixed with adhesive plaster to given places 2 cm. apart in a line parallel with the underlying muscle fibers. The earth connection was made with an electrode placed in the hypochondrium region along the linea semilunaris.

Recording and its conditions. An 8-channel electroencephalograph of Sanei Measuring Instrument Manufacturing Company (time constant: 0.05 second) was used. All the records illustrated in the present paper were obtained with the ink-writer incorporated in the apparatus. In all the figures, the action potential height of 100μV was represented by the length of a vertical bar, and the time interval of 1 second by the length a horizontal bar.

Simultaneously with electromyograms, the respiration curve was recorded, which was obtained by electric leading of the movement of the thorax at the level

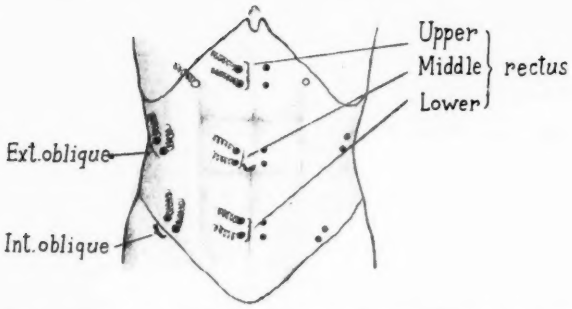

Fig. 1. Placing of surface electrodes on the skin above the various abdominal wall muscles.

of the fifth rib.

Results

With the healthy humans, electromyograms were recorded at various postures and during various movements. Patterns of the action potentials thus obtained were qualitatively the same in all the experiments with all the subjects, diferring only quantiatively according to cases.

A. Patterns of activity in the supine posture

1) *At rest.* Figure 2 shows the patterns of activity in the supine position a) When the abdominal wall was released from strain by the drawing-up of the legs, and b) when the legs were extended. In both experiments, a rhythmic spike-like discharge less than 100μV in amplitude appeared in the lead from the upper part of the recti, which was interpreted to be the mingling of electrocardiographic changes. There was seen a sparse base line fluctuation (less than 5 μV in amplitude) in the leads from the upper, middle, and lower parts of the recit. The external and internal obliques also gave discharges, which were denser and of larger amplitude (5–10 μV) than the three parts of the recti. By reference to the respiration curve it was found that these discharges were not affected by respiration.

The next experiment was to see, on the subject in the positions with the legs drawn up or extended, effects of applying a gentle finger pressure to various parts of the abdominal wall in a manner as in the defense test ; but there were seen no changes in action potential (Fig. 3). A sudden and rough application of finger pressure, on the other hand, evoked in the internal and external obliques more or less condensed but transitory discharges 15 and 25 μV in amplitude respectively.

2) *Head raising* (Fig. 4). When the head was slightly raised (to an angle of 10°) from the horizontal position, there appeared dense discharges in the recti (amplitude: 70–100, 30–50, 20–30 μV in the upper, middle, lower parts respectively) and a slightly increased discharge (amplitude: 5–15μV) in the external obliques ; but no definite discharge was recognized in the internal

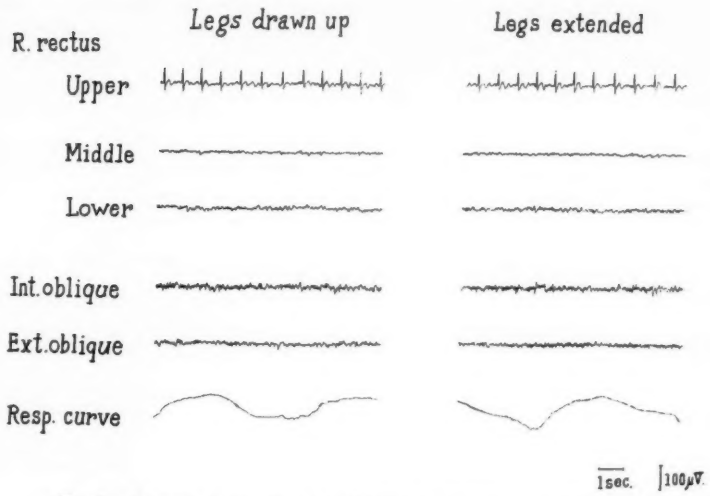

Fig. 2. Patterns of activity of the abdominal muscles of the subject in the supine position at rest.

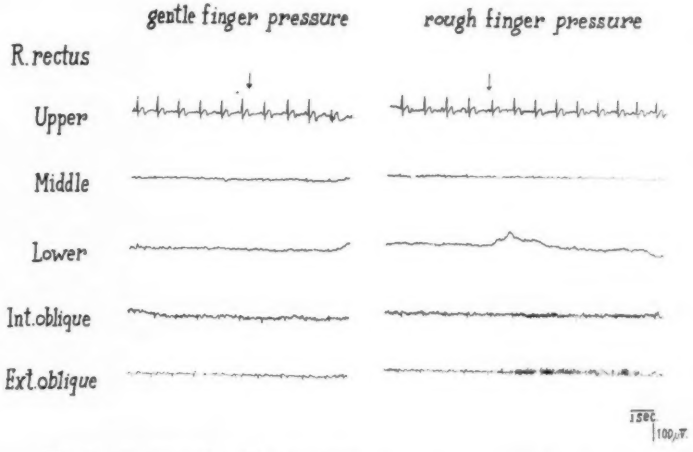

Fig. 3. Patterns of activity for applying finger pressure to the abdominal wall in the supine position.

obliques.

On the other hand, a much higher raise of the head (to an angle of 30°) produced dense and remarkably increased discharges in the recti (amplitude: 130μV or higher in the upper and middle parts, 90–110 μV in the lower) and

dense and increased ones in the internal and external obliques (amplitude: 20–30 and 80–110 μV respectively).

3) *Deliberate increase of intra-abdominal pressure* (Fig. 5). When the subject increased his intra-abdominal pressure deliberately, there occurred an increase in discharge. This increase was only slight in the recti (amplitude: 5–15, 5 or less, 10–30 μV in the upper, middle, lower parts respectively), while in the internal and external obliques it was very remarkable both in frequency and amplitude (amplitude: 50–90, 70–120 μV respectively).

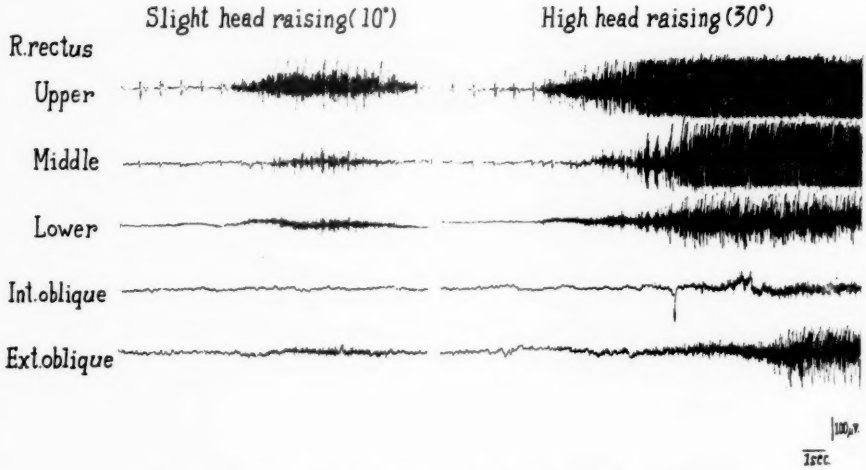

Fig. 4. Patterns of activity for raising the head from the supine position.

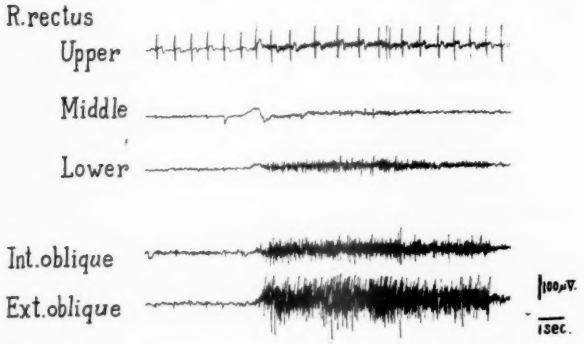

Fig. 5. Pattern of activity for deliberate increase of intraabdominal pressure in the supine position.

4) *Deep breathing* (Fig. 6). During deep breathing there appeared, in

conformity with the expiratory phase in the respiration curve, definitely increased discharges in the upper recti (10–15 μV), in the internal obliques (15–25 μV), and in the external obliques (especially remarkable and dense; 30–50 μV).

During the inspiratory phase also there were seen discharges in the recti (less than 5 μV in all the three parts) and in the internal and external obliques (5–10 μV); but no definite differences were noticed between them and those during quiet breathing.

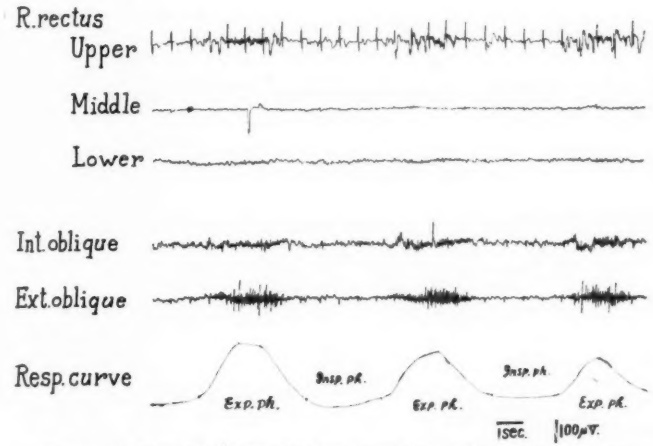

Fig. 6. Pattern of activity for deep breathing in the supine position.

5) *Voicing* (Fig. 7). In the experiment in which the subject was made to produce the sound [a :], no discharge was noticed when the volume of the voice was small; when the volume was large, however, the voicing produced a discharge of either of the following two types: (A) dense discharges in the upper recti, and internal and external obliques (70–130, 50–110, 20–30 μV respectively) and sparse discharges in the middle and lower recti (10–20 μV or less), or (B) dense discharges in the internal and external obliques (100–160, 25–40 μV respectively) but no definite ones in the three parts of the rectus. These differences in pattern of activity between types A and B seemed to be due in some cases to individual differences; but in other cases, both types occurred on the same subject.

6) *Coughing* (Fig. 8). Light coughing evoked discharges of higher amplitude in the upper recti and the internal and external obliques (90–110, 80–100, 90–120 μV or more, respectively) and those of lower amplitude in the middle and lower recti (20–40, 15–20 μV respectively). Strong coughing, on the other hand, produced dense discharges of higher amplitude in all cases (130 μV or more, 90μV or more, 70 μV or more in the upper, middle, lower recti respectively; 100 μV or more, 150 μV or more in the internal and external obliques respectively).

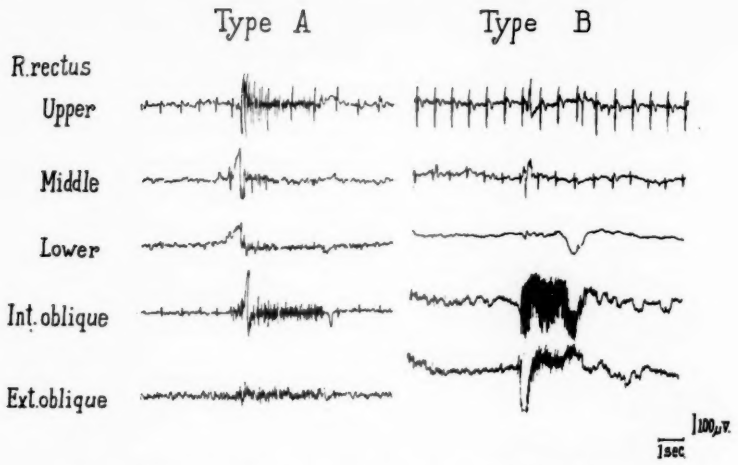

Fig. 7. Patterns of activity for producing the sound in the supine position.

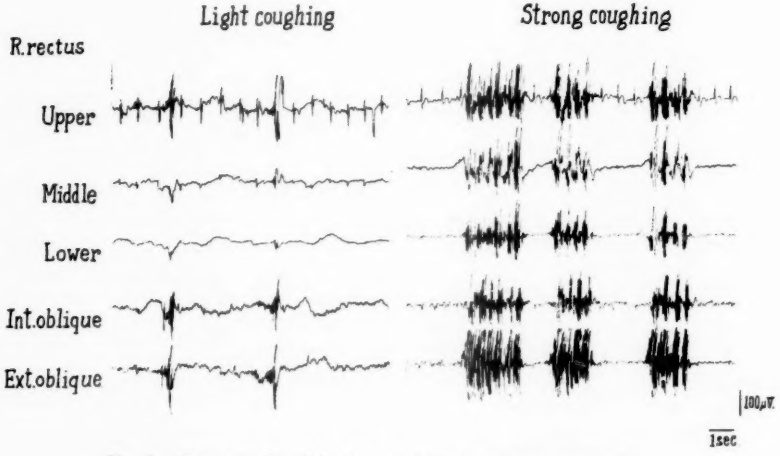

Fig. 8. Patterns of activity for coughing in the supine position.

7) *Raising one leg from the supin position* (Fig. 9). When the right lege was raised from the horizontal position, electromyograms on that side showed dense and definitely increased discharges in all cases (50–100, 20–40, 20–60, 40–80, 50–90 μV in the right upper, middle, lower rectus and the internal, external obliques respectively). When the left leg was raised from the horizontal position, electromyograms on the opposite side showed dense and increased discharges in the right upper and lower rectus and the right external oblique (15–

30, 150–30, 30–70 μV respectively) and dense but only slightly increased discharges in the right middle rectus and internal oblique (10–15, 10–20 μV respectively).

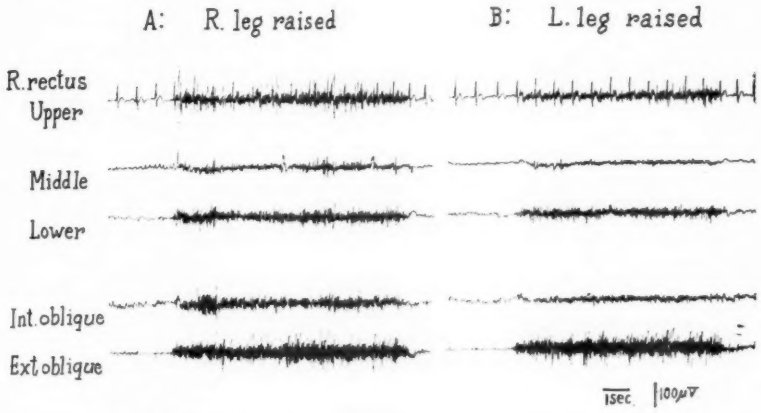

Fig. 9. Patterns of activity for raising one leg from the supine position.

B. Patterns of activity in the standing posture

1) *At rest* (Fig. 10). In the pattern of activity of the subject standing at rest, increased discharges from the internal obliques 10–25 μV in amplitude were to be noted. The other muscles showed no difinite differences in discharge from the corresponding ones of the subject in the supine position at rest.

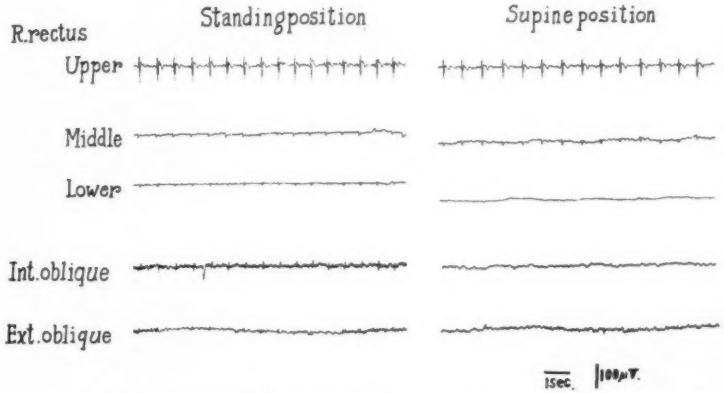

Fig. 10. Comparison of the patterns of activity of the same subject at rest in the standing and supine positions.

2) *Forward leaning of the trunk and the returning* (Fig. 11). The only change to be noted at the time of a gentle forward leaning of the trunk was a slight

increase in discharge of the external obliques (10–25 μV). During the returning movement, there were seen increased discharges in all the muscles except the internal obliques (15–25, 10–20, 60–100, 25–50 μV in the upper, middle, lower recti, and the external obliques respectively).

3) *Backward leaning and the returning* (Fig. 11). During the movement of gentle backward leaning of the trunk, increased discharges appeared in all the muscles (140 μV or more in the three parts of the recti ; 25–120, 30–130 μV in the internal and external obliques respectively). During the returning movement the discharges suddenly decreased and returned to their initial states at rest.

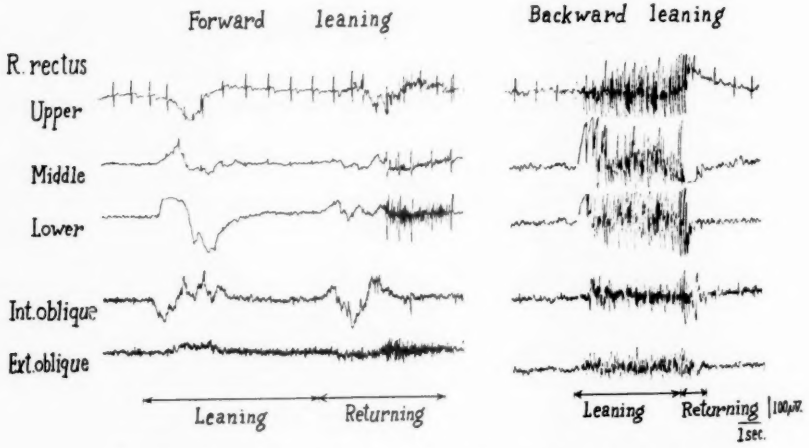

Fig. 11. Patterns of activity for flection and extension of the trunk from the standing position at rest.

4) *Sideward flection of the trunk* (Fig. 12). During the movement of side bending of the trunk to the right, there were observed slightly increased discharges in the rectus and internal oblique on that side (10–15, 5–10, 10–30, 10–20 μV in the right upper, middle, lower rectus, the right internal oblique respectively), while moderately increased discharges appeared in the right exteranl oblique (30–50 μV).

When the trunk was bent to the left, the muscles on the opposite (right) side showed the following discharges: A slightly increased discharge in the right rectus (10–20, 5–10, 15–25 μV in the upper, middle, lower parts respectively), a moderately increased discharge in the right internal oblique (30–70 μV), and a highly increased discharge in the right external oblique (120 μV or more). Thus, it will be seen that the right external and internal obliques showed definitely increased discharges during side bending of the trunk to the left, as compared with the changes seen during the bending to the right.

C. Patterns of activity in various postures

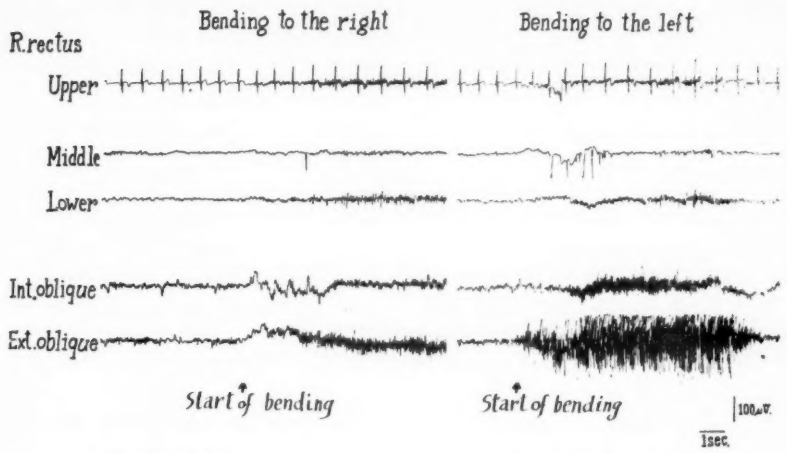

Fig. 12. Patterns of activity for side bending of the trunk from the standing position at rest.

Figure 13 shows the patterns of activity in the same subject for the following various positions: a) in the supine position, b) sitting, legs extended, c) sitting upright in Japanese fashion, d) sitting cross-legged, e) in the standing position, and f) sitting on a chair.

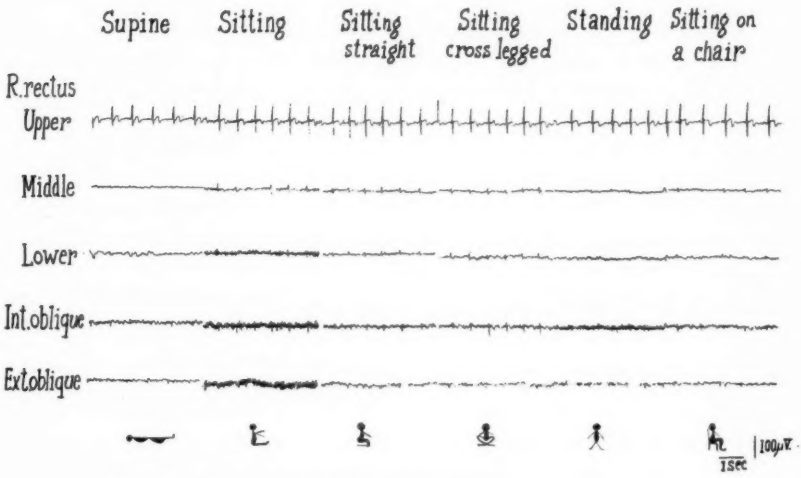

Fig. 13. Patterns of activity in various postures.

In the position of sitting with the legs extended, slightly increased discharges appeared in the lower recti and the internal and external obliques (5–15, 10–20,

15–25 μV respectively), as compared with the discharges in the supine position. In the positions of sitting in Japanese fashion, sitting cross-legged, and sitting on a chair, the patterns of activity were essentially the same as that observed in the supine position.

Discussion and Conclusions

Electromyographic studies with surface electrodes on the abdominal wall muscles in man have been reported by Floyd,[2] Maki *et al.*,[3] Nakano *et al.*,[4] Nanjo,[5] Komiyama *et al.*,[6] and other workers. Particularly, Floyd[2] made an extensive electromyographic study of patterns of activity of the abdominal wall muscles in various postures and during various movements.

In the present work, I also made similar study, in which electromyographic records were taken with 50 healthy subjects at various postures and during various movements, by means of surface electrodes placed at given positions on the skin above the upper, middle, and lower parts of the rectus, and the internal and external obliques on each side.

In the supine position, whether with the legs drawn up or with the legs extended, no discharge due to muscular activity was recognized. A sudden, rough application of strong pressure to the abdominal wall muscles in the supine position caused a weak, transitory discharge in the internal and external obliques, though an application of gentle pressure produced no discharge.

As to the raising of the head from the horizontal position, a slight raise (to an angle of 10°) caused strong discharges in the upper recti and weaker ones in the middle and lower recti and external obliques, while in the experiment of raising the head much higher (to an angle of 30°) the discharges in the above muscles were much denser and more remarkable, the internal obliques also showing weak but increased discharges. From these results it will be concluded that the muscles involved in the movement of raising the head are the upper, middle, and lower parts of the recti and the external obliques in order of decreasing importance.

From the pattern of activity for deliberate increase of intra-abdominal pressure (Fig. 5), the external and internal obliques (particularly the former) were found to be involved in this action to a great degree.

By an experiment of deep breathing it was confirmed that deep breathing evoked definite discharges in the internal and external obliques during the expiratory phase (Fig. 6).

When the sound [a :] was being produced there were seen two different patterns of activity (Fig. 7) : A pattern in which the internal and external obliques and the upper recti were mainly involved; and a pattern in which the external and internal obliques were involved to a great degree.

As to the pattern of activity for coughing, the upper recti and the external and internal obliques were involved in light coughing, while all the muscles including the middle and lower recti (which were relatively inactive in light coughing) showed a very high activity (Fig. 8).

When one leg was raised from the supine position, all the muscles on that side were found to be involved in the movement, while those on the opposite side showed weak discharges with the exception of the external oblique, the discharge of which was quite strong (Fig. 9).

In the standing position at rest, the internal obliques alone seemed to be involved, showing a weak discharge. When the trunk was leaned forward from the standing position at rest, the external obliques alone seemed to be involved in the movement, showing a weak discharge. In contrast with this, during the returning movement from leaning, all the muscles except the internal obliques were involved in the movement (Fig. 11). When the trunk was leaned backward from the standing position at rest, there were seen a strong discharge in the recti and weak ones in the external and internal obliques. When the trunk was extended again, all the discharges suddenly decreased until the intial states were recovered (Fig. 11).

When the trunk was being bent sideward, the internal and external obliques on both sides, and especially the external oblique on the opposite side, were found to be mainly involved in the movement (Fig. 12).

Of the other various postures studied, that of sitting with the legs extended gave weak discharges in the lower recti and the external and internal obliques; the postures of sitting straight in Japanese fashion, sitting cross-legged, and sitting on a chair evoked no psecial discharge in all the muscles examined.

From these results it may be concluded that in movements such as raising of the head and backward leaning of the trunk, which are accompanied by flection of the trunk, the recti are involved mainly. On the other hand, in those cases in which the abdomen is strained (movement or postures such as deliberate application of a strain to the abdomen, deep breathing, voicing, and sitting with the legs extended), the internal and external obliques are primarily involved. These conclusions are in general agreement with Floyd's opinions[2] that the recti come into action when a movement against gravity is being made, and that the external and internal obliques are related with the intra-abdominal pressure.

Floyd[2] reports very few cases in which a slight spontaneous discharge was recognized at rest; but its relation with respiratory influences is not clear in his paper. In the present work, not in a single case was noticed a definite discharge that was suspected of action currents of the abdominal muscles at rest. Generally, my findings agree with what he already con-

firmed except for the following points: There was a tendency in the rectus that a discharge was apt to appear in the upper part of the muscle and was more difficult to appear in the lower and middle parts in increasing order; and the amplitude of discharges observed was smaller than that reported by him.

Summary

With 50 normal humans, electromyographic study by means of surface electrodes was made on the activity of the abdominal wall muscles in various postures and during various movements.

1. In the supine position, whether with the legs drawn up or with the legs extended, no discharge due to muscular activity was recognized. Gentle pressure applied to the abdominal wall also did not evoke discharge.

2. During the movement of flection and extension of the trunk (such as head raising from the supine position and backward leaning in the standing position) there was seen a remarkable discharge mainly in the recti. In those cases in which the abdomen was strained (such movements as deliberate application of a strain to the abdomen in the supine position, deep breathing, voicing, and sitting with the legs extended), remarkable discharges were seen in the external and internal obliques.

3. The electromyographic patterns of activity for sitting straight in Japanese fashion, sitting cross-legged, sitting on a chair were essentially the same as that obtained in the supine position at rest. In the standing position at rest, on the other hand, there was seen a discharge 10–15 μV in amplitude in the internal obliques; in the position of sitting with the legs extended, slightly increased discharges in the internal and external obliques (10–20, 15–20 μV respectively) and in the lower part of the recti (5–15 μV), as compared with the discharges in the supine position at rest.

4. There was seen in the recti a tendency that discharge was apt to appear in the middle, lower, and upper parts in order of increasing readiness.

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Descending Spinal Pathway Subserving the Adrenaline Secretion of the Adrenal in Dogs

By

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Tournade, Hermann, Malmejac and Jourdan[1] studied the descending spinal pathway of secretory fibers to the adrenal medulla. In their experiments the method of suprareno-jugular anastomosis was employed and the hemisection of the spinal cord was made at the level of Th2. The faradization of the spinal cord above the site of hemisection resulted in an increase in the adrenaline secretion from the ipsilateral as well as from the contralateral adrenal gland. From these results it was concluded by them that the adrenalino-secretory fibers partly passed down directly the spinal cord and partly decussated. Evidence given by them, however, is to be brought into question, since the possibility of the current spread to just below the site of section cannot be ruled out.

Here, the adrenalino-secretory action of insulin hypoglycemia is recalled. The augmentation of adrenaline secretion induced by insulin hypoglycemia is well established. It was proved by Sato, Kanowoka and Ohmi[2] that this augmentation was wholly abolished by the transection of the spinal cord at the lower cervical segments. And it was concluded that insulin hypoglycemia increased the adrenaline secretion in acting on the center situated above the level of the section.

In the present investigation we have examined in the first place whether the findings of Sato *et al.* can be duplicated by our hands. Our success in the duplication led us to apply insulin hypoglycemia to the study of the descending spinal pathway. Experiments were performed in an attempt to know how the hemisection of the spinal cord at the lower cervical level acts on the secretion of adrenaline after insulin from each of both adrenal glands.

Experimental

Methods

Unanesthetized dogs were experimented on. The adrenal venous blood was sampled by means of the lumbar route method of Satake, Sugawara and Watanabe.[3] The adrenaline content of the blood specimens was estimated by the rabbit intestinal segment method, adrenaline (Sankyo) being taken as the standard. The blood sugar content was determined by the method of Hagedorn and Jensen. Insulin was injected intravenously in a dose of 7 units per kg. of body weight.

In the present investigation 2 sets of experiments were performed.

In the first set, the total section of the spinal cord was made at the level of C7-C8 under ether anesthesia and 2 days later the experiments were done without anesthetizing the animals. The adrenal venous blood from the left gland was sampled.

In the second set, the dorsal spinal roots of ThII-L3 were cut under ether anesthesia in order to perform experiments without anesthetizing or evoking any pain. About 3–14 weeks later the hemisection of the spinal cord was made in the left side at the level of C7-C8 under ether anesthesia and on the following day or 2 days later the experiments were performed without anesthesia. In these dogs the adrenal venous blood specimens were sampled from each of both adrenal glands. At the termination of experiments the spinal cord from the lower cervical to the upper thoracic segments was removed and the site of hemisection was verified histologically.

Results

(1) *Experiments in dogs subjected to the spinal cord transection.*

Experiments were performed in 2 dogs. The results are presented in Table I.

In Exp. 1 the blood sugar content before insulin was 0.097–0.099%. After the insulin injection it decreased step by step. At 1 hour it reached 0.059%. Two hours after injection it was measured as 0.048% and 30 minutes later it was 0.039%. The adrenaline secretion rate before insulin was 0.01 μg. per kg. of body weight per minute. After insulin there was no increase in the adrenaline secretion. It was measured as 0.005–0.008 μg.

In Exp. 2 the blood sugar level before insulin was 0.077–0.079%. On receiving insulin it decreased and 0.034% was reached 2 hours after insulin. The rate of adrenaline secretion before insulin was 0.01–0.02 μg. per kg. per minute. After insulin it remained unaltered.

Thus it was proved that the acceleration of adrenaline secretion causable by insulin hypoglycemia was wholly prevented by the total section of the spinal cord at the lower cervical segments.

TABLE I

Adrenaline Secretion Rate after Insulin in Dogs Subjected to the Spinal Cord Transection

No. of experiment	Body weight and sex	Time (minute) before or after injection	Blood sugar content (%)	Adrenaline secretion rate (μg.) per kg. per minute	Heart rate (per minute)	Respiration (per minute)	Body temperature (°C.)
1	15.9 kg. ♂	Before 20	0.099	0.01	82	16	37.0
		5	0.097	0.01	82	20	36.9
		Injection of insulin (7 units per kg.)					
		After 20	0.072	0.008	84	22	36.9
		40	0.068	0.007	80	18	36.6
		60	0.059	0.007	78	20	36.4
		90	0.057	0.007	78	20	36.2
		120	0.048	0.008	70	22	35.2
		150	0.039	0.005	60	20	34.4
2	17.5 kg. ♂	Before 25	0.077	0.02	58	19	31.1
		5	0.079	0.01	60	22	31.2
		Injection of insulin (7 units per kg.)					
		After 60	0.045	0.02	58	29	31.7
		90	0.039	0.02	50	31	31.8
		120	0.034	0.02			31.5
		150	0.039	0.02	46	23	

(2) *Experiments in dogs subjected to the spinal cord hemisection.*

Experiments were performed in 4 dogs. The data are displayed in Table II. In examining histologically the site of hemisection after each experiment, it was found that in Exps. 5 and 6 the section of the left lateral half of the spinal cord was complete and in Exps. 3 and 4 a small portion of ventral column in the left side was remained uninjured.

In Exp. 3 the blood sugar content before insulin was 0.120–0.124%. After insulin injection it decreased noticeably. Forty to 120 minutes after injection it was determined as 0.042–0.048%. The last specimen taken 2.5 hours after insulin showed 0.037%. The initial rate of adrenaline secretion from the right as well as from the left adrenal gland was found to be 0.02 μg. per kg. per minute. After insulin it was accelerated and reached 0.11 μg. in the left gland and 0.10 μg. in the right gland, respectively, the maximal acceleration being noted within 1.5 hours after injection. Then it decreased step by step. During the whole course no noticeable difference

was found in the adrenaline secretion rate between the left and the right glands.

In Exp. 4 the blood sugar content before insulin was 0.115%. It decreased gradually after insulin. One hour and a half after injection there was a low blood sugar level such as 0.045% and half an hour later it descended further, 0.038% being reached. The adrenaline secretion rate

TABLE II

Adrenaline Secretion Rate after Insulin in Dogs Subjected to the Spinal Cord Hemisection

No. of experiment	Body weight and sex	Time (minute) before or after injection	Blood sugar content (%)	Adrenaline secretion rate (μg.) per kg. per minute		Heart rate (per minute)	Respiration (per minute)	Body temperature (°C.)
				Right gland	Left gland			
3	13.5 kg. ♀	Before						
		25	0.124	0.02		160	16	38.2
		20	0.120		0.02			
		10	0.122	0.02		176	18	38.3
		5			0.02			
		Injection of insulin (7 units per kg.)						
		After						
		20	0.078	0.04		184	16	38.3
		25			0.06			
		40	0.044	0.07		194	16	38.1
		45			0.04			
		60	0.048	0.08		192	20	37.8
		65			0.06			
		90	0.042	0.10		184	22	37.6
		95			0.11			
		120	0.044	0.08		186	18	37.5
		125			0.06			
		150	0.037	0.03		176	18	37.3
		155			0.03			
4								
	12.8 kg. ♂	Before						
		20	0.115	0.03		136	16	38.3
		15			0.02			
		10	0.115	0.03		138	14	38.3
					0.02			
		Injection of insulin (7 units per kg.)						
		After						
		30	0.070	0.08		144	16	38.2
		35			0.08			
		60	0.054	0.08		152	18	38.1
		65			0.06			
		90	0.045	0.11		156	20	37.9
		95			0.05			
		120	0.038	0.43		98	8	37.8
		125			0.19			
		150	0.036			142	12	37.9

5	14.0 kg. ♂	Before 20	0.106	0.04				
		15		0.04	0.02	148	16	37.2
					0.02			
			Injection of insulin (7 units per kg.)					
		After 30	0.086	0.12				
		35			0.12	124	16	37.4
		60	0.063	0.11				
		65			0.10	184	15	36.6
		90	0.043	0.11				
		95			0.16	190	16	36.4
		120	0.031	0.32				
		125			0.11			
		135				176	14	36.6
6	18.0 kg. ♂	Before 35			0.01			
		30	0.119	0.03		166	13	39.0
		15			0.01			
		10	0.120	0.02		182	13	38.5
			Injection of insulin (7 units per kg.)					
		After 20			0.01			
		25	0.085	0.06		184	15	38.7
		40			0.04			
		45	0.081	0.06		182	14	38.6
		60			0.06			
		65	0.072	0.14		178	14	38.6
		90			0.04			
		95	0.061	0.13		190	17	38.5
		120			0.01			
		125	0·057	0.06		182	16	38.1

before insulin was 0.02 μg. per kg. per minute in the left and in the right adrenal gland. About 30 minutes after insulin the adrenaline secretion from the left as well as from the right adrenal gland was somewhat accelerated and reached 0.08 μg. One hour after insulin it was 0.06 μg. in the left and 0.08 μg. in the right gland, respectively, and 30 minutes later the secretion rate of the left gland was measured as 0.05 μg., whereas that of the right gland was 0.11 μg. Half an hour later it was found to be 0.19 μg. in the left and 0.43 μg. in the right gland, respectively.

In Exp. 5 the initial blood sugar content was 0.106%. After insulin the level of blood sugar descended and 0.031% was reached within 2 hours after injection. The adrenaline secretion rate before insulin was 0.02 μg. per kg. per minute in the left gland and 0.04 μg. in the right gland, respectively. Half an hour after injection it was found to be 0.12 μg. in the left as well as in the right gland. Half an hour and one hour later the secretion rate of adrenaline of the left gland was determined as 0.10 μg. and 0.16 μg., respectively. And that of the right gland was measured as 0.11 μg. About 2 hours after insulin the rate of adrenaline secretion of the left gland was 0.11 μg., whereas that of the right gland was 0.32 μg.

In Exp. 6 the blood sugar content before insulin was found to be 0.119–0.120%. After insulin it decreased gradually. About 2 hours after insulin it reached 0.057%. The adrenaline secretion rate before insulin was 0.01 μg. per kg. per minute in the left gland and 0.02–0.03 μg. in the right gland. After insulin it increased and reached 0.06 μg. in the left and 0.14 μg. in the right gland, the maximum being found within one hour after insulin.

Discussion

Evidence provided by Sato *et al.* was confirmed in the first series of experiments. Insulin hypoglycemia was proved to induce an increase in the adrenaline secretion by acting the adrenalino-secretory center situated above the lower cervical segments. Hence, if the descending spinal pathway of adrenalino-secretory fibers runs directly without crossing, the hemisection of the spinal cord may nullify the increase in the adrenaline secretion causable by insulin hypoglycemia only of the ipsilateral adrenal. And if it decussates completely below the lower cervical segments, the increase may be observed only in the ipsilateral gland. However, neither of them has turned out to be the case. In our experiments the adrenaline secretion was found to be increased by insulin hypoglycemia in the ipsilateral as well as in the contralateral gland, though it was mostly somewhat less pronounced in the former than in the latter.

Thus, it may be reasonable to conclude that in dogs the descending spinal pathway of the adrenalino-secretory fibers decussates partially in the spinal cord below the cervical segments.

Summary

The experiments were performed in unanesthetized dogs. The adrenal venous blood was sampled by the lumbar route method of Satake and others and was assayed for adrenaline by the rabbit intestinal segment method. The following results were obtained: The increase in the adrenaline secretion causable by insulin hypoglycemia was found to be wholly abolished by the total section of the spinal cord at the level of C7–C8. In dogs subjected to the hemisection of the spinal cord at the same level as above, the adrenaline secretion was increased by insulin hypoglycemia in the contralateral as well as in the ipsilateral adrenal gland.

It is concluded that the descending spinal pathway of the adrenalino-secretory fibers decussates partially below the lower cervical segments.

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Development and Dissipation of Brightness Induction in the Human Retina

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Introduction

The phenomenon of color induction has been investigated by Motokawa[1),2)] and his co-workers mostly in the dark-adapted retina. However, very little is known about brightness induction or black-white induction, probably because experiments are more difficult owing to rapid dissipation of induction in the light-adapted retina. In the present experiment, development and dissipation of brightness induction were investigated under various adaptation states of the retina. As was shown by Motokawa[2)], two kinds of brightness induction, direct and indirect, can be distinguished; the direct induction can be proved by exposing one and the same retinal area successively to a black and a bright stimulus which raise the electrical excitability of the retina above the control excitability following the bright stimulus alone. The indirect induction is measured by exposing two retinal areas in juxtaposition successively to an inducing bright stimulus and a test bright stimulus, or simultaneously to a black and a bright stimulus. The detail of experimentation will be described together with the results.

Experimental

Method

To control the degree of light adaptation, the illumination on a white screen standing vertically in front of the subject's eye was varied from 0.005 to 25 lux by means of suitable filters. The reflection factor of the white screen was about 0.84. The color temperature of 6 illuminating lamps was about 1,770°C. The distance between the screen and the subject's eye was always 40 cm. In the middle of the screen, there was a small opening, which was covered from behind by a cylindrical pipe of 20 cm. in length and 5 cm. in diameter. The inside surface of the pipe was dead black all over, so that the opening appeared as a black patch on the white screen. The opening could be closed and opened

manually by means of a cover which was as white as the screen.

The pipe mentioned above served not only to furnish a black stimulus, but also to furnish a localized light stimulus in the following way: The surface at the bottom of the pipe opposite the opening consisted of opaque glass, whose transmission factor was about 0.78. When the opaque glass was illuminated from behind, the opening looked like a bright patch on the white screen. Thus, a dark and a light stimulus could be applied in succession, and the effect of successive induction upon the electrical excitablity of the eye was investigated. When a dark and a light stimulus were to be presented in juxtaposition at the same time, an annulus of black velvet was used around the bright test patch (See Fig. 2).

For testing electrical excitability, a single constant current pulse of 0.1 sec. in duration, was applied to the eye through a pair of silver electrodes of 2×1.5 cm^2. in size, with a cathode on the forehead slightly above the eyebrow and an anode on the homolateral temple of the subject. The contact of the electrodes with the skin was secured by the use of electrode paste. A constant resistance of 10 kilo-ohms was inserted in series in the stimulating circuit to reduce the effect of variation in resistance of the tissue. The procedure of threshold determination was the same as a standard one described by Kohata *et al.*[3)]

Results

1) *Dependence of the resting electrical excitability on the intensity of adapting light*

At first, the influence of the intensity of adapting light upon the resting excitability of the retina was investigated. The resting excitability of the dark-adapted retina was measured and then the retina was light-adapted to a general illumination of certain intensity for 10 minutes or longer. The measurement of excitability was carried out again in that adaptation state. The electrical excitability of the resting retina increased naturally in accordance with the intensity of the adapting light. As can be seen in Fig. 1, the excitability as expressed in percentage of the dark excitability increased linearly with logarithmic intensity of adapting light in the range from 0.01 to 10 lux. In Fig. 1, E_{od} and E_o denote resting excitabilities (reciprocals of electric thresholds) of the dark-adapted and of the light-adapted retina respectively. But the excitability approached a steady level at about 25 lux.

2) *Brightness induction in the light-adapted retina*

In the light-adapted retina, supernormal excitability was seen after exposure of the retina to a white light stimulus stronger than the adapting light; the degree of supernormality was greater with increasing intensities of the white light stimulus. The time course of excitability had a maximum at 2 sec. after the end of exposure just as in the dark-adapted retina. The broken curve in Fig. 2 shows the time course of an excitability curve for a white stimulus, a white circular patch of 10 mm. in diameter or 1°30′ in visual angle and of 100 lux, the adapting light being 0.9 lux. When the stimulus patch was enclosed by a black ring the stimulating effect was increased, and in consequence the excitability curve increased in height, as if the stimulus intensity had been increased (see curves

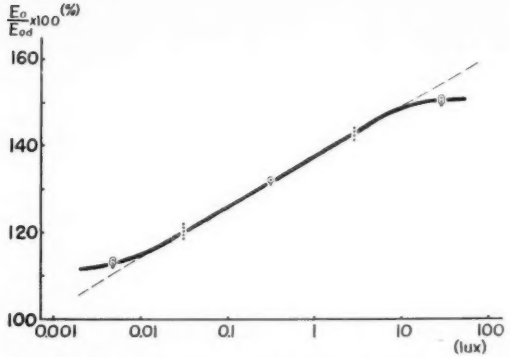

Fig. 1. Dependence of resting excitability on intensity of adapting light. Ordinates: Excitability in percentage of that of dark-adapted retina. Abscissae: Intensity of adapting light in lux (logarithmic scale).

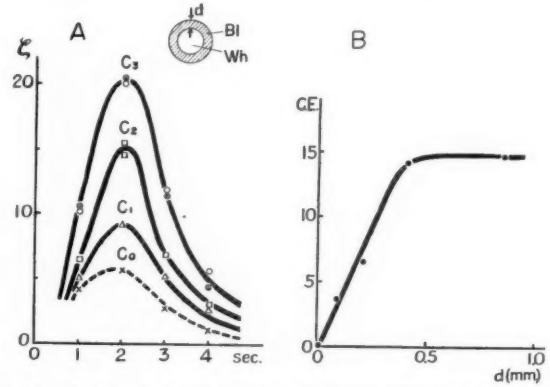

Fig. 2. A: Time course of ζ, i.e. percentage increase in electrical excitability after removal of white light, Wh. Co: Control curve for Wh alone. C_1, C_2 and C_3: Curves for Wh enclosed by a black ring of 2, 5 and 10 mm. in width respectively. Result obtained by Wh with a black ring (Bl) of 20 mm. in width is represented by C_3, too. Adapting light: 0.9 lux. B: Contrast effects (C.E.) versus width of black ring (in retina). C.E.: Difference between C_1, C_2 or C_3 and Co.

C_1, C_2, and C_3 in Fig. 2). The curves C_1 and C_2 were obtained for different widths of the black ring, namely 2 and 5 mm. respectively. Curve C_3 represents the results obtained with black rings of 10 and 20 mm. in width. Those maximum differences between C_o and C_1, C_2 or C_3 were defined as the "black-white contrast effect" or the "intensity of brightness induction." The C.E. (contrast effect) so defined was found to be almost independent of the duration of the patch exposure, unless the duration was too short or too long.

The C.E. was generally a function[2),5)] of distance from the inducing stimulus ; it decreased exponentially as the distance increased. In Fig. 2 B, the C.E. was represented as a function of the width of the black ring reduced to the value in the retina. As can be seen in Fig. 2 B, the C.E. reached a steady level at a distance of about 0.4 mm. This fact indicates that the effective part of the black stimulus surrounding a bright stimulus is confined to a inner zone of 10 mm in width (0.4 mm. in retina).

In the second series of experiment, the indirect brightness induction was measured by the other methods in the light-adapted retina. A semicircular inducing patch, I, of 10 mm. in diameter and a circular test patch, Wh, of 1 mm. in diameter were arranged as is shown in the inset of Fig. 3. The illumination of both patches was 100 lux, and the intensity of adapting light was 0.9 lux. The patches, I and Wh, were each presented for 2 sec. successively, and the C.E. was measured. In this case, one may suppose that such high supernormal excitability was obtained as the result of the summation of the effects evoked by I and by Wh. But such assumption was obviously untenable, because isolate presentation of an I patch which was slightly increased in area caused no such remarkable supernormality as above. The observed increase of electrical excitability caused by successive stimuli, I and Wh, above that caused by Wh alone may be interpreted as follows : The inducing stimulus, I, induced in the surrounding area blackness induction, which has the same effect as a black stimulus applied directly upon this area. We know that the same retinal area exposed successively to a black and a white stimulus shows remarkable electrical excitability (direct brightness induction). Hence the simultaneous effect of a bright stimulus, I, is equivalent to the direct effect of a black stimulus. The result is illustrated in Fig. 3, in which the C.E. was plotted against the distance, d, between both retinal images of I and Wh. It is likely that black induction started at the

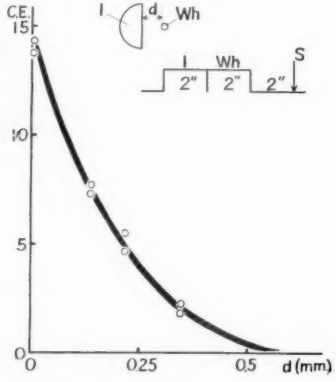

Fig. 3. Spread of brightness induction. I : Inducing white patch succeeded by white test light. Wh, as is shown in inset where S denotes electrical stimulus. Distance, d, is expressed in terms of lateral distance in retina.

image of semicircular patch was propagated into the surrounding area and raised the sensitivity to the test stimulus, Wh. From this experiment it can be seen that the induction effect spreads over a distance of about 0.5 mm. in the adaptation state used in this experiment.

3) *Dissipation of brightness induction*

Retinal induction remains for some time after removal of the inducing stimulus. In the following series of experiment, the mode of dissipation of the direct induction caused by a black stimulus was investigated under various degrees of light adaptation. The eye was exposed first to a black patch and then to a white test patch with a variable interval, τ, between them. The C.E. was measured at 2 sec. after removal of the white patch. The diameter of each patch was 10 mm., and each duration of exposure was 2 sec. The illumination of the white patch was 100 lux. The result obtained is represented diagrammatically in Fig. 4, in which level of light adaptation, L.A., interval, τ, and C.E.

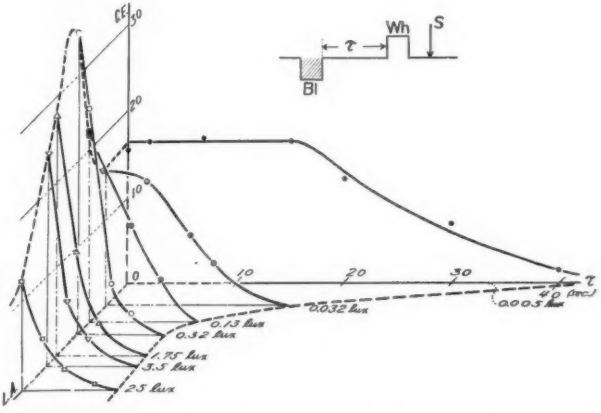

Fig. 4. Decay of induction caused by black stimulus as a function of intensity of adapting light (L.A.). τ: Interval between inducing black stimulus (Bl), a circular spot of 1 cm. in diameter, and white test light (Wh).

are represented three-dimensionally as x, y and z respectively. For 0.005 lux of light adaptation, the C.E. was constant within 15 sec. of τ, but decreased exponentially as τ was lengthened further (see the continous curve marked by dots). In this case the C.E.-τ curve was of the same form as those obtained in the dark-adapted retina by Motokawa.[4] At a light adaptation level of 0.032 lux, both constant and decreasing parts of the C.E.-τ curve were shorter than above. In cases of 0.32, 1.75, 3.5 and 25 lux of light adaptation, the constant part could not be seen any more, but the C.E. decreased from the start exponentially as τ increased, and the C.E. disappeared in about 8 sec. The broken curve on the τ-L.A. plane (see Fig. 4) indicates how the survival time of black induction depends on the adaptation state of the retina.[5),7),8)] On the other

hand, the broken curve on the C.E.-L.A. plane (see Fig. 4) indicates how the intensity of black induction at $\tau=0$ depends on the degree of light adaptation. As can be seen in this curve, the optimum level of light adaptation lies at about 1 lux.

4) *Further studies on the optimum adaptation level for brightness induction*

From the above-mentioned result it is apparent that there is an optimum light-adapted state[7),8)] for the development of black-white contrast effect. In the following series of experiment it will be shown that the optimum level of adaptation differs according to the intensity of the white test light. The white test light was 100 lux in the preceding case. So the measurements of the C.E. were carried out with white test lights of 10 lux and 1000 lux. The results are shown in Fig. 5, in which the C.E. is plotted against the degree of light adap-

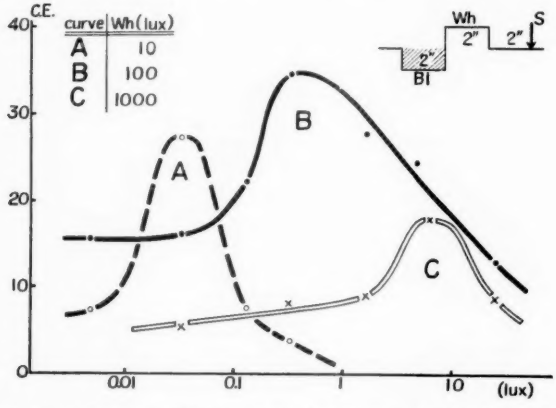

Fig. 5. Influence of adapting light on successive black-white induction. Abscissae : Intensity of adapting light (logarithmic scale). Curves A, B and C were obtained for the test white patches of 10, 100 and 1,000 lux respectively.

tation. Curves A, B and C were obtained for the white test lights of 10, 1000 and 1,000 lux respectively. It can be seen in this figure that the intensity of each optimum adapting light is about one percent of the test light. The similar results were obtained by Motokawa[2)] in 1949, but his experiment was carried out only in the range of the adapting light from 0.006 to 0.077 lux, and white test light of 84 lux was used.

5) *Effects of a black stimulus on electrical excitability*

In all the experiments mentioned above the induction effect of a black stimulus was measured by combinating it with a white light and an electric stimulus. From these experiments it has been elucidated that a black stimulus has an effect to enhance the stimulating effect of a succeeding white stimulus or that of a similar stimulus presented simultaneously in juxtaposition. The question arises as to what is the direct effect of a black stimulus on the electrical excitability of the retina. Therefore in the following experiment, changes of electrical excita-

bility following a black stimulus of 2 sec. were investigated without intervening a white test light. The adaptation level was varied from 0.005 to 25 lux. The results obtained are summarized in Fig. 6. Supernormal excitability after the black stimulus could be seen only in a limited range of adapting light from 0.005 to 0.14 lux, and in these cases the crest time of the excitability curve was 2.0 sec. In the range of adapting light from 0.32 to 25 lux, the excitability was generally subnormal and the maximum of the excitability was not found at 2.0 sec.

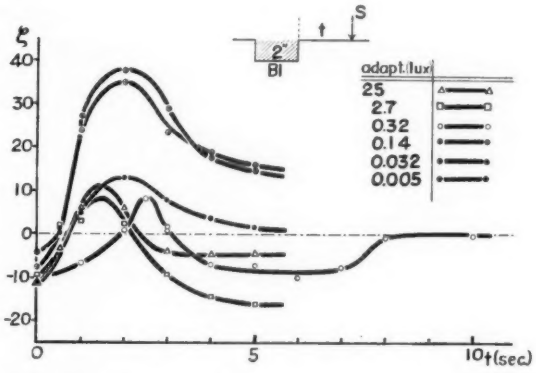

Fig. 6. Time courses of electrical excitability after exposure to a black patch of 1 cm. in diameter in various degrees of light adaptation.

In the next series of experiment, the electrical excitability was measured during presentation of the black stimulus, instead of after removal of it. As shown in Fig. 7, the electrical excitability was subnormal or ζ-values were negative[9] during the presentation of the black patch at all adaptation levels. The

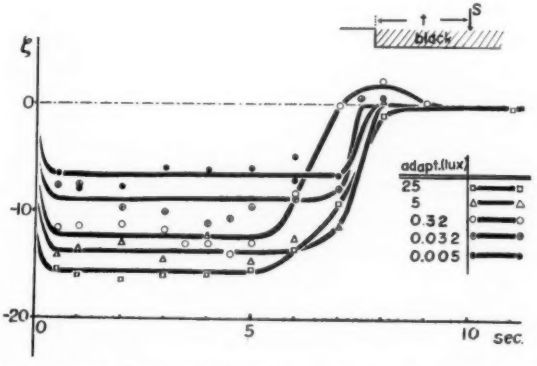

Fig. 7. Subnormal excitability-time curves during exposure to the same black patch as in Fig. 5.

negativity of ζ was greater for higher adaptation levels. It is to be noted that the subnormal excitability remained constant for about 5 sec., and that the excitability recovered spontaneously from the subnormal state towards the normal level at the further stage of the continous stimulation with the black patch. This phenomenon was observed not only at the fovea, but also at the periphery 20° from the fovea.

Discussion

The induction effect of a black stimulus is a function of various factors, such as the adaptation state of the retina, the intensity of the white test light, etc. The present experiment and a preceding one by Motokawa[2] have established a rule that an optimum induction effect of a black stimulus can be obtained when the intensity of adapting light is about one percent of that of the white test light. This rule would be of great use for further experiments on brightness induction. It is conceivable that the adapting light is a factor controlling the intensity of the stimulus; the blackness of the stimulus must be higher as the intensity of adapting light is stronger, although allowance has to be made for irradiation which counteracts black-white contrast. If the inducing effect of the black stimulus is based on its blackness it is to be expected that the effect increases with the intensity of adapting light. This is certainly the case so far as the intensity of adapting light is below a certain level. Above the critical intensity, the inducing effect of the black stimulus decreases, thus giving rise to an optimum (see Fig. 5). This depressant effect of adapting light upon induction may be interpreted in two ways: 1. As the intensity of the white test light is fixed in the above-mentioned experiment, the effect of the test light decreases when the intensity of adapting light approaches that of the former. 2. The dissipation of the induction effect is facilitated by adapting light (see Fig. 4). The first interpretation is supported by the fact that the optimum intensity of the adapting light is raised by the use of stronger test light (see Fig. 5), but the second scarcely holds for the present experiment in which the induction was tested immediately after removal of the inducing black stimulus.

Summary

The effect of combined stimuli, black and white, upon the electrical excitability of the human retina was investigated under various conditions of light adaptation.

1. The electrical excitability of the resting retina increases in accordance with the degree of light adaptation when the intensity of adapting light is below 25 lux.

2. The effect of a circular bright stimulus to increase electrical ex-

citability is enhanced when the patch is enclosed by a black ring (simultaneous induction).

3. Dissipation of induction is facilitated by adapting light.

4. There is an optimum adaptation state for brightness induction. The optimum intensity is about one per cent of the intensity of the white light following a black stimulus.

5. A subnormal electrical excitability is seen for the first 7 sec. during presentation of a black patch in the light adapted eye. The electrical excitability returns then to the initial level spontaneously even when the black stimulus continues.

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